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SYMPATHETIC CONTROL OF ENERGY METABOLISM  
AND HEART RATE IN THE SHEEP

by



FREDERIC LEROY HAYS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE  
OF MASTER OF SCIENCE

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

OCTOBER, 1968



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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Sympathetic Control of Energy Metabolism and Heart Rate in the Sheep" submitted by Frederic Leroy Hays, B.Sc.(Ag), in partial fulfilment of the requirements for the degree of Master of Science.





## ABSTRACT

Experiments were conducted to study the involvement of the sympathetic nervous system in sheep during eating and during acute cold exposure. Propranolol, a beta-adrenergic blocking agent, was used to inhibit the effect of sympathetic stimulation. In the first experiment, propranolol at 0.25, 0.5 and 1.0 mg/kg was used to inhibit beta-adrenergic activity in sheep with fleece during feeding and during exposure to  $-30^{\circ}\text{C}$ . Subsequently the effects of propranolol were tested on sheared sheep exposed to  $-30^{\circ}\text{C}$ . Finally the effects of total pharmacological cardiac blockade with atropine (0.05 mg/kg) and propranolol (0.5 mg/kg) were tested during eating and during direct cardiac nerve stimulation.

Metabolic rates were determined from respiratory exchange and heart rates were determined by electro-cardiography. Beta-adrenergic blockade alone markedly reduced cardio-acceleration during cold stress but not during eating. Propranolol had no effect on energy expenditure during eating. Increased energy expenditure during mild cold stress was reduced by propranolol at 1.0 mg/kg.

During severe cold stress cold-acclimated sheep increased heat production to a level about 25% higher than warm-acclimated sheep. Winter-acclimatized sheep exposed to a similar air temperature increased heat production less than either of the other groups.

The results indicate that the thermal insulation was greatest in these animals. Propranolol at 1.0 mg/kg inhibited maximal heat production during this severe cold exposure by about 6% in the warm-acclimated sheep and 12% in the cold-acclimatized sheep. No effect was observed in the winter-acclimatized sheep.

Cardio-acceleration following direct stimulation of the stellate ganglion in anaesthetized sheep was effectively inhibited for at least



two hours by propranolol at 0.5 mg/kg. Atropine at 0.05 mg/kg inhibited the direct effects of stimulation of the vagal efferents to the heart.

Resting heart rate in conscious sheep following pharmacological blockade with propranolol and atropine was shown to increase from about 60 beats/min to about 100 beats/min which is presumably the intrinsic rate of the normal heart.

The heart rate of thyroidectomized sheep did not change following pharmacological denervation with isolation. Moreover, eating did not significantly change the rate of isolated hearts in euthyroid or thyroidectomized sheep.



## ACKNOWLEDGEMENTS

The author wishes to thank Dr. L. W. McElroy, Chairman of the Department of Animal Science, for placing the facilities of the Department at his disposal. The guidance and encouragement provided by Dr. A. J. F. Webster, Assistant Professor of Animal Physiology, throughout the course of this study and his constructive criticisms and suggestions for the improvement of this manuscript are gratefully appreciated.

The author is also indebted to Dr. R. T. Hardin, Associate Professor of Poultry Genetics, for assistance with the statistical treatment of the data. Sincere thanks are extended to Mr. N. Arbon and Mr. B. Pringle for invaluable assistance throughout the experiments and to Miss Sandra Popik and Miss Lenora Israelson for typing of this manuscript.

Financial assistance for this project was provided from the National Research Council in the form of an Assistantship.





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## I. INTRODUCTION and REVIEW OF LITERATURE

### INTRODUCTION

In order for the life processes of homeotherms, such as sheep, to function at maximal efficiency a constant body temperature must be maintained. The reference point for body temperature is usually taken as the temperature of the deep tissues in the body core. In reality, however, body temperature can show a considerable variation from the periphery to the absolute center of the animal. Mean body temperature is vulnerable, at all times, to fluctuation due to energy demand of body functions such as in exercise or to changes in ambient temperature.

The body is continuously subjected to a multiple of changes which must be integrated to maintain a state of equilibrium. Homeostatic control was defined by Cannon (1929) as "the factors which operated in the body to maintain uniformity". The study of homeostasis is simplified by considering separate systems such as the nervous system or the circulatory system, in isolation while remembering that none are separate entities to themselves.

The autonomic nervous system has been shown to be responsible, in part, for mechanical and chemical modulation of the internal environment in response to different demands of certain body functions. This study is concerned with the extent of autonomic involvement in the regulation of heart rate and energy exchange in sheep in response to external stimuli of cold and feeding. Some of this work (Experiment 1) has been reported already by Webster and Hays (1968).

With world population estimated at 6.5 billion by 2000 A.D.,



or about two times that of 1968, animal production will have to increase to 583% (as compared to the base year 1958-59) if all people are to be fed minimal energy and protein requirements (Byerly, 1966). Animal product will have to be produced more efficiently in existing, developed agricultural areas and areas not now acceptable for animal production will be exploited. Such progress requires a greater understanding of livestock responses in various normal and extreme situations.

Quantitative knowledge about specific demands of energy for all facets of possible livestock husbandry are required to estimate and predict possible efficiency of production under varied conditions. The energy cost of feeding in the sheep has been rather disputed in past reports. In order to clarify this, information is required on the physiological changes associated with eating in the ruminant. The Alberta winter undoubtedly provides conditions which can be unfavorable for livestock production. Fundamental studies of the effects of cold stress on sheep will help to increase efficiency of production in these areas.



## REVIEW OF LITERATURE

### A. Receptor Sites in the Sympathetic Nervous System

During the last seventy years much has been achieved in elucidating the interrelationships between the actions of the sympathetic nervous system and the adrenal medulla. Olivier and Schäfer (1895) were the first to disclose that the effects of injected adrenal medullary extract caused an increase in blood pressure. Sympathetic nerve stimulation was regarded by Cannon and Bacq (1931) as an outflow of neurohormone from nerve excited smooth muscle. This neurohormone was labelled sympathin and was to be the basis of much controversy for the next thirty years.

Dale (1906) was one of the first to recognize the receptor context of sympathetic nerve stimulation. His classical paper illustrated the inhibitory effects of ergot alkaloids in sympathetic transmission at the myoneural junction and termed these sites the "receptive mechanisms for adrenaline".

Certain paradoxical effects of sympathetic stimulation and adrenaline <sup>1</sup> and noradrenaline injection were explained by Ahlquist (1948) who suggested two types of adrenergic mechanisms: alpha and beta receptors. Functions ascribed to alpha receptors include vasoconstriction, increased uterine contractility, and intestinal relaxation. Beta receptors have been associated with vasodilation,

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<sup>1</sup>Adrenaline is the European and English name used to identify the adrenal medulla amine extract. Adrenalin is a registered trade name in the United States and the generic name epinephrine is commonly used. Noradrenaline and norepinephrine are common used names for levarterenol.





bronchiodilation and myocardial stimulation (Ahlquist, 1967).

It is now quite conclusive that, in the mammal, noradrenaline is the primary sympathetic neural transmitter and adrenaline the primary adrenal medullary hormone (Kopin, 1966). Noradrenaline has been shown to mediate non-shivering cold thermogenesis and increase the rate and force of contraction in heart muscle. This catecholamine is considered primarily a potent alpha-adrenergic agent with weak beta-adrenergic properties except on the heart while adrenaline has both alpha and beta-adrenergic properties (Furchgott, 1967).

Sympathomimetic drugs such as isoproterenol have been used to isolate and demonstrate the effects of adrenergic receptors. Isoproterenol is a drug similar to adrenaline and noradrenaline in chemical structure and has strong beta adrenergic tendencies with weak alpha-adrenergic properties (Ariens, 1967).

Much of this work has been done with the help of adrenergic receptor blocking agents. These blocking agents are drugs which, in some way, inhibit the action of nerve stimulation or hormones at the receptor sites (for example, the neuromuscular junctions). Ideally, they should provide total block of these effects without themselves stimulating the mechanisms or liberating endogenous catecholamines (Blinks, 1967; Furchgott, 1967). They should be effective in small doses so that gross physiological side effects are limited. The ergot alkaloids used by Dale (1906) were such agents and since this time various other specific adrenergic blocking agents have been isolated and incorporated in receptor research. Phenoxybenzamine (Furchgott, 1967) and phentolamine (Antonis et al., 1967) have been used as



effective specific alpha-adrenergic blockers and dichloroiso-proterenol (DCI), pronethalol and more recently propranolol<sup>2</sup> (Black et al., 1964) as specific beta receptor blocking agents. The first specific beta-adrenergic blocking agent to be developed, DCI, exhibited complicating sympathomimetic properties (Powell and Slater, 1958; Moran and Perkins, 1958).

It was estimated (Black, Duncan and Shanks, 1965; Shanks, 1966; McInerny, Gilmour and Blinks, 1965) that propranolol had a therapeutic ratio which was ten fold greater than pronethalol. Propranolol did not produce the side effects in man found with pronethalol of lightheadedness, uncoordination and nausea and vomiting (Black et al., 1964).

Ledsome, Linden and Norman (1965) demonstrated that in dogs intravenous infusion of propranolol (0.5 mg/kg) would prevent from 75 - 90% of the change in heart rate caused by reflex changes in the sympathetic nervous system. It had been suggested (Black, Duncan and Shanks, 1965; Shanks, 1966) that propranolol was devoid of intrinsic sympathomimetic activity. Blinks (1967) using in vitro kitten and guinea pig cardiac tissues subjected to propranolol treatments showed that at all concentrations used ( $10^{-10}$  to  $10^{-4}$  M in the bath), little sympathomimetic effect was found. Occasionally increases in rate or force followed administration of concentrations between  $10^{-8}$  and  $10^{-6}$  M but these were slight. Both pronethalol and propranolol

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<sup>2</sup>Propranolol (I.C.I. 45, 520; Inderal; 1-iso propylamino - 3 - (1 naphthyloxy) - 2 - propanol hydrochloride (Black et al., 1964).



exhibited simple competitive antagonisms very similar to the atropine-acetylcholine antagonism at muscarine receptors (Brink and Hutchison, 1965; Nakano and Kusakari, 1965; McNery, Gilmour, and Blinks, 1965).

Different types of beta receptor mechanisms have been suggested in different species and different tissues. Rall and Sutherland (1962) indicated that the beta-adrenergic receptor concept was closely related to the adenyl cyclase enzyme system. It has been shown (Rall and Sutherland, 1958; Sutherland and Robison, 1966 and Mayer et al., 1967) that in many tissues activation of adenyl cyclase to catalyze ATP to adenosine 3', 5' AMP (cyclic AMP) brings about a formation of the active phosphorylase a, an enzyme required for the conversion of glycogen to glucose phosphate through an activation of phosphorylase kinase. Adenyl cyclase has been found in all animal cells examined except non-nucleated erythrocytes (Sutherland and Robison, 1966). Liver, skeletal muscle and heart tissue are very dependent on this enzyme for glycogenolysis and the increased production of glucose. Adrenaline, ACTH and glucagon will stimulate activation of phosphorylase in many tissues but with different sensitivities (Sutherland and Robison, 1966; Namm and Mayer, 1968). Robison (1967) suggested that the enzyme adenyl cyclase was actually the beta-adrenergic receptor.

#### B. Autonomic Regulation of Heart Rate

The efferent links in the nervous control of the heart consist of sympathetic adrenergic fibers and vagal cholinergic fibers.





In all cases where specialized adjustments are required, increased or decreased sympathetic tone may occur; decreased or increased vagal tone may co-exist with changing sympathetic control or may be perhaps the prevalent factor. The two different branches of the autonomic nervous system have been shown (Robinson et al., 1966) to regulate both the force of contraction of the myocardium (intropic effect) and the rate of pulsation of the heart muscle (chronotropic effect). Stimulation of the sympathetic nerves resulted in an increased heart rate and force of contraction while parasympathetic stimulation brought about temporary heart blockade.

Starling, as reported by Chapman and Mitchell (1965), disclosed his theories on the relative importance of neural control of heart rate and stroke volume. His main concept of cardiac control was that diastolic distensibility was the main mechanism regulating ventricular stroke volume. The force of myocardial contraction was supposedly primarily dependent upon the resting length of the myocardial fibers. He reported that an intrinsic myocardial response was the main controlling factor and that autonomic reflex control acted on it to facilitate adaptation to stress.

Loewi (1921) demonstrated that vagal stimulation of a donor frog heart provided a perfusion fluid which slowed the heart of a recipient frog heart. This he called "Vagusstoffe". He also discovered that an accelerator substance similar to adrenaline was liberated into the perfusate upon stimulation of the accelerator nerves. Atropine has been shown to competitively inhibit the





muscarine-like effect of acetylcholine produced by stimulation of a parasympathetic nerve such as the vagus. It has been shown that vagal stimulation and acetylcholine infusion slows the pacemaker activity by increasing potassium permeability of the membrane (Hoffman, 1962).

The effect of exercise on cardiovascular function has long been a topic of interest (Braunwald, 1966). Graser and Meek (1914) indicated that there were two different types of exercise tachycardia. the immediate increase in heart rate due to exercise and the prolonged residual increased heart rate due to continued exercise. More recently Donald and Samueloff (1966), working with dogs, suggested that the availability of noradrenaline in the blood stream during exercise contributed to the increase in heart rate and the ability of the animal to perform maximal exercise. Robinson et al (1966) suggested that with man light exercise tachycardia was due primarily to the withdrawal of parasympathetic stimulation while increased sympathetic activity became progressively more important in cardiac acceleration.

It has been possible to study some control mechanisms of the heart by cardiac denervation. The greatest disadvantage of this technique is possible interruption of other important functions of the body (i.e., respiration) which could impair metabolism indirectly. These could, perhaps, be avoided by more precise surgical techniques. Donald and Shephard (1963) denervated the hearts of dogs. Their technique involved ablating the sympathetic network of the heart and severing both vagi.



Adrenalectomy was also included to eliminate the effect of catecholamines of adrenomedullary origin. Mild exercise was performed on a treadmill and heart rate, cardiac output, stroke volume and oxygen consumption measurements were recorded. Cardiac output increased with exercise in both the denervated and the control animals. In the denervated preparations this increase was brought about, primarily, by an increase in stroke volume ( ml blood flow/heart beat). Some exercise tachycardia was evident during severe exercise but it was not as pronounced as in the normal controls; the increase being about 25% above resting values in the denervated dogs compared to an increase of 100% in the normal controls. Adrenalectomy did not have an effect on the change in heart rate patterns or on the plateau values reached. It was found, however, that the heart was more sensitive to noradrenaline after denervation. This is characteristic of denervation (Trendelenburg, 1966).

Heart sympathectomy did not affect the ability of dogs to perform maximal amounts of exercise (Donald and Shephard, 1964). Maximal exercise was evidenced when the animals could no longer run on the treadmill and fell exhausted. Similar results were indicated by propranolol treated dogs (Donald and Samueloff, 1966). However, the cardiac denervated dogs in the same study could not maintain the maximal amount of exercise demonstrated before treatment with propranolol. It was suggested that maximal effort required the cardio-stimulant effect of the cardiac sympathetic nerves and noradrenaline released into the circulation from nerve endings elsewhere in the body. Decreasing the effect of either of these decreased the



capacity of these animals for maximal effort (Donald and Milburn, 1968). Recent work by Ashkar, Stevens and Houssay (1968) suggested, however, that cardioacceleration during exercise in chronic denervated dogs required catecholamines of adrenomedullary origin. Noradrenaline released at post-ganglionic nerve endings away from the heart was not, in their opinion, related to this tachycardia observed during exercise.

Pharmacologically denervated hearts have illustrated similar results in man (Cumming and Carr, 1967; Robinson et al., 1966). This work involved infusion (i.v.) of atropine (0.03 mg/kg) and propranolol (5 mg) followed by exercise on a bicycle ergometer. Such pharmacologically isolated hearts resemble a heart-lung preparation (Jose, 1966). Although an exercise tachycardia was evident, cardiac output was due primarily to an increased stroke volume compared to the increased heart rate in normal patients. Pharmacological denervation with such drugs tends to isolate the heart from hormonal actions of the entire autonomic nervous system and the adrenal medulla. Propranolol decreased the total heart rate and cardiac output before and after high levels of exercise with a mean fall in arterial oxygen content after exercise (Robinson, et al., 1965; Cronin, 1967; Epstein et al., 1965). The decrease in cardiac output of these treated animals (a drop of 20%) after exercise was compensated by increased extraction of oxygen from the blood.

By studying force-velocity relationships of the hearts of man, Sonnenblick (1965) indicated that in the normal state exercise tends to increase profoundly the contractile properties of the



myocardium. This was attributed to the effect of the sympathetic nervous system. However, beta-adrenergic blockade by propranolol inhibited any increase in force of myocardial contraction but heart rate as well as cardiac output did increase.

Exercise tachycardia was demonstrated in isolated hearts attached to exercising dogs (Donald and Samueloff, 1966). Propranolol again was used to block any adrenergic hormonal effects and the test dogs were exercised on a treadmill. Tachycardia of the isolated heart and the heart of the untreated exercising dog was proportional to the work performed. Propranolol tended to abolish the increase in heart rate of the isolated heart but did not affect the donor dog heart significantly in any way. This tends to suggest that exercise tachycardia is not due to some blood-borne compound, such as catecholamines, but could possibly be due to some intrinsic property of the heart.

Tachycardia has been illustrated by increasing the stretch on isolated sinus node tissue and increasing the perfusion pressure for in vitro heart preparations (Lange et al., 1966; Pathak, 1966). A tendency for tachycardia blockade was illustrated with DCI (dichloroisoproterenol - Section A) in the perfused heart preparation while adrenaline and atropine in the perfusate did not show any effects on stretch induced-acceleration. A possible mechanism for this tachycardia may be related to increased permeability of sodium across the membrane (Lange et al., 1966).

Webster (1967) recorded similar increases in heart rate in sheep when energy expenditure was increased to a comparable degree







by cold exposure or by eating which suggests that the mechanisms controlling heart rate were similar in both instances. The present study was designed, in part, to explore the authenticity of that assumption.

### C. Sympathetic Regulation of Energy Metabolism

A recent review (Himms-Hagen, 1967) deals extensively with the sympathetic control of metabolism. Only specific points relevant to this study are mentioned below. It is well documented that endogenous catecholamines and sympathetic neural stimulation can increase metabolic responses of muscle, fat tissue and smooth muscle. A quantitative release of free fatty acids (FFA) from subcutaneous adipose tissue in dogs was evident following electrical sympathetic nerve stimulation of physiological strength. It was concluded that sympathetic control could mobilize energy for the animal within a few minutes.

Alpha and beta-adrenergic receptors are both thought to be active in the hyperglycemic response to adrenaline in man (Pinkington et al., 1962). Adrenaline infusion increased blood glucose and lactate levels in normal man (Antonis et al., 1967). Propranolol abolished the increase in lactate but not glucose following adrenaline infusion; phentolamine (an alpha-adrenergic blocking agent - Section A) had no effect on either parameter. However, infusion of both alpha and beta blocking agents prevented any increase in blood glucose or lactate. In patients with dietary induced ketosis propranolol alone was able to prevent an increase in glucose and lactate



after adrenaline infusion. From this the authors concluded that propranolol in the normal patient inhibits glycolysis in muscle; the rise in blood glucose resulted from glycogenolysis in the liver which must have been mediated by alpha-adrenergic receptors. Phentolamine in the normal patient blocked this alpha-receptor glycogenolysis of the liver but stimulation of glycolysis in muscle produced lactate and subsequent gluconeogenesis in the liver with this lactate as the substrate. Ketotic patients who were unable to exhibit glycogenolysis in the liver could not, therefore, increase blood glucose after beta blockade. Thus it was suggested that in man, beta-adrenergic receptors are situated in the muscle and alpha receptors in the liver. Despite this, no well defined inter-tissue or inter-species conclusions have as yet been reached which permit a generalized classification as to the role of alpha-and beta-adrenergic receptors in the control of energy metabolism.

Sympathetic control of hepatic glycogenolysis by adrenaline was suggested by Ezdinli et al. (1968). Assuming that the central nervous system contains strategically located centres sensitive to blood glucose they sectioned the spinal cord of anesthetized dogs. Arterial blood glucose levels dropped. Adrenaline induced hyperglycemia was greatly inhibited in the dogs with spinal section compared to normal dogs while glucagon induced hyperglycemia was not significantly inhibited. It was suggested that adrenaline induced hepatic glycogenolysis was mediated through activation of the hyperglycemic brain center with effective pathways reaching the pancreas via the sympathetic nerves. Thus, they concluded that glucagon and



adrenaline may act together to form a very efficient mechanism for support of blood glucose level.

Metabolic responses to stimuli such as cold exposure and exercise have been shown in some way to involve the sympathetic nervous system. Hsieh, Carlson and Gray (1957) realized that the sympathetic nervous system had some effect in the control of metabolism of rats exposed to cold but that the calorogenic role depended on the history of cold exposure in the animal. At that time, noradrenaline was recognized as a calorogenic agent (Hsieh and Carlson, 1957). Sympathetic stimulation or catecholamine infusion in rats, however, only produced a marked response after they had been previously subjected to cold exposure.

Exercise induced increases in rate of lipid and carbohydrate metabolism have been demonstrated in man, dogs and rats (Himms-Hagen, 1967). Muir, Chamberlain and Pedoe (1964) measured the effect of beta blockade on free fatty acid, (FFA) and carbohydrate metabolism in man. Measurements were conducted at rest and during exercise. It was shown that the rate of mobilization of FFA found during exercise could be inhibited by pronethalol. This beta-adrenergic blocking agent also lowered the resting FFA level which suggested that sympathetic tone could possibly mobilize these fatty acids continuously. Beta-adrenergic blockade did not change blood glucose levels before or after exercise.

Issekutz et al., (1966) have suggested that plasma bound FFA are an important fuel for muscle during exercise in dogs. However, the actual mechanism of control was not studied.



The total aerobic metabolism of carbohydrates in the heart of man and dog accounts for approximately 35% of the total myocardial oxygen consumption (Bing, 1965). It seems quite doubtful (Mayer, Williams and Smith, 1967) that adrenaline-induced glycogenolysis has any physiological significance in the normal, aerobically, respiring mammal heart. This tends to indicate that some other non-carbohydrate fuel is utilized by the heart. Paul and Issekutz (1967) demonstrated that the energy supply for the dog heart during short hard bursts of exercise was about 20-30% FFA while for prolonged work 70-90% of the energy was from this source. Oxidation of plasma glucose in either case was quite minor and contributed not more than 10-15% of this expenditure. It was shown (Mayer et al., 1963) that the inotropic effect on the myocardium could occur before activation of phosphorylase although it was apparent that cyclic AMP was involved. An increase in mechanical activity of the heart was attributed to changes in these adrenaline nucleotide concentrations but the actual mechanism were not known (Williamson, 1966).

The present study is concerned with sympathetic and other mechanisms controlling energy metabolism and heart rate. No attempt has been made, however, to investigate the biochemical mechanisms involved in the control of these functions. It is fundamental that the possible avenues of biochemical control discussed above be finally resolved.

#### D. Responses of Sheep to Cold

The study of temperature regulation in homeotherms and







especially temperature regulation under the conditions of cold exposure has been expanding since before the last world war. The physiological effect of cold exposure is defined as the tendency of the environment to cause a change in the individual (Carlson et al., 1953) and is termed environmental cold stress. This change in the homeotherms which alters physiological function to maintain core temperature is termed strain. The amount of cold stress to which the animal can be subjected is a function of the basic amount of heat produced (Swift, 1932), the type and amount of insulation (Webster and Park, 1967), the amount of adaptation to the environment (Glaser, 1950) and the amount of energy expended in muscular exercise. The thermoneutral range of the animal is that ambient temperature range in which there is neither an increased metabolism to maintain body temperature nor increased metabolism to dissipate heat.

The lower limit of the thermoneutral range is generally referred to as the critical temperature. As air temperature falls below the critical temperature, metabolic rate must increase to meet the rising thermal demand of the environment. If an animal is allowed to eat more than a maintenance ration or is allowed to exercise or has additional amounts of insulation, the critical temperature will be lower and the thermoneutral range possibly greater than with a basal state (Kleiber, 1961).

Much of this cold environmental work has been with the white rat and man. There are, however, indications that species variation for many of the body functions exists and so it is necessary to



realize that cold stress may create species specific physiological functions.

Engineering models of control mechanisms have been used to explain the effect of central thermal control (Hardy, 1961; Bligh, 1966). It is now accepted that a well defined temperature regulation centre exists in the hypothalamus but some ignorance prevails as to the effect of other controlling mechanisms such as peripheral receptors and extrahypothalamic temperature receptors in the brain and in other deep body tissues. It has been shown that input signals from peripheral receptors (Zotterman, 1953) activate the central effect of thermal regulation to cold exposure through a rate controlling process (Hardy, 1961).

In the long term, the heat production of an animal must be equal to its total heat loss. Heat loss or heat dissipation refers to heat lost by convection, conduction, radiation, evaporation, heat lost when expiring air and loss in urine and feces.

Maximal metabolic responses to cold stress have been defined as "Summit metabolism" (Alexander, 1962). If heat loss is faster than heat can be produced, the body temperature will fall and death may ensue. Consequently, a measure of summit metabolism must depend on the rate of fall in body temperature and duration of the measurement. Although, the summit metabolism for lambs of three to five kg was about 17 kcal/kg/hour or about five times basal metabolism, it has been suggested that the increased size of the adult sheep may provide a summit metabolism per kg weight which is higher. Adaptation to the cold may increase this metabolic value.



Following a sudden drop in ambient temperature or increased wind speed in the cold, an initial compensatory increase in metabolic rate is experienced which is greater than the normal equilibrium value. This rise in heat production or "metabolic overshoot," has been noted in man and sheep (Joyce and Blaxter, 1964). During this metabolic overshoot in sheep, heat production is much greater than heat loss. In order to remain in a homeostatic state, the animal must decrease heat production until thermal equilibrium is again attained. This rate of increasing metabolism and the time required to reach a level of thermal equilibrium of oxygen consumption, and rectal and skin temperatures after sudden exposure to a cold environment is a function of the severity of the acute cold stress and the length of fleece which determines its rate of onset (Webster, 1966).

It was recognized that shivering was a form of muscular work which the body incorporated to increase warmth (Hemingway, 1963; Glickman et al., 1967). A comparison of energy expenditure during cold exposure and exercise in the warm acclimated dog (Chatonnet and Minaire, 1966) suggested that shivering could account almost entirely for cold thermogenesis.

It has been shown, however, in many species including rats, dogs, rabbits and cats (Hemingway et al., 1964; Carlson, 1966) that adaptation to cold involves a transition from shivering as the main source of incremental heat to non-shivering thermogenesis (NST) (i.e., no measure of electrical activity of the muscle contractile units) (Sellers, Scott and Thomas, 1954). Nonshivering thermogenesis can be divided into two components: (a) obligatory NST and (b)





regulatory NST (Hsieh et al., 1966). Obligatory NST is that minimal amount of heat which is produced to maintain the animal. This is highly correlated to the amount of circulating free thyroid hormone (i.e., thyroid activity). Cold adaptation demonstrates an increased basal metabolic rate and possibly increased ability to utilize body reserves (Slee and Sykes, 1967). Regulatory NST is the incremental heat production stimulated by an environment lower than the critical level. This non-shivering thermogenic effect is by far the more important part of homeostosis at low environmental temperatures where maximal heat production is warranted and is seen to be additive (for the white rat) to shivering thermogenesis (Jansky, 1966).

The calorogenic effect of catecholamines from the sympathetic nervous system and adrenal medulla in some animals is well accepted (Carlson, 1966; Himms-Hagen, 1967) but, as already discussed, the actual biochemical mechanisms are as yet not well defined. Noradrenaline infusion in warm adapted white rats resulted in dose dependent mobilization of free fatty acids and increase in metabolic rate (Hsieh et al., 1966). This amine not only increases calorogenesis but also controls the circulatory adjustments for prevention of heat loss (i.e., constriction in various circulatory beds).

When the activity of sympathetic nerves was reduced by inhibition or neural noradrenaline synthesis, rats were still able to increase thermogenesis during cold-acclimation by increasing adrenal medullary adrenaline (Johnson, et al., 1966). It was suggested that this is but one of the compensatory mechanisms that may play a part in maintenance of homeothermy.





In the past some conflict in terminology has arisen between acclimation and acclimatization. The meanings based on definitions proposed by Hart (1961) are: (1) acclimatization refers to the physiological changes induced in organisms by a "complex of factors such as seasonal and climatic changes" and (2) acclimation proposes changes induced by a "single environmental factor, as in controlled experiments".

Usually rodents undergoing acclimation have been fed ad libitum with voluntary feed intake increasing in the cold. Since this could be considered as one of the parameters of cold acclimation, additional energy could complicate determination of other parameters. Slee and Sykes (1967) fed sheep two planes of ration at a constant level in a cold environment (+8 C) and found that acclimation, as demonstrated by the rate of ability of these animals to adjust to the cold, was evident in both groups although those animals fed at the low place showed a lesser ability to adapt.

During the initial reaction to cold stress in warm-acclimated man increases were recorded in thyroid hormone, cortisol, catecholamines, ACTH, TSH, systolic and diastolic blood pressure and blood sugars (Bigelow and Sidlofsky, 1961; Suzuki et al., 1967). Thyroidectomy or adrenalectomy decreased the initial elevation in metabolic rate and and it has been suggested that metabolic adaptation to cold demonstrates some interaction between thyroid hormone and hormones of the sympathetic nervous system. Andersson et al., (1967) has shown that thyroidectomized goats under cold stress increased noradrenaline secretion whereas only a minor increase in adrenalin<sup>e</sup> secretion was reported.



Hypothyroid animals reacted differently with noradrenaline secretion increasing only slightly above the level of the controls but the adrenaline secretion reached a level more than five times greater. Although thyroid hormones are of importance in nonshivering thermogenesis (Carlson, 1960) sympathetic responses are undoubtedly one of the main contributing factors (Hsieh, Carlson and Gray, 1957; Johnson, Schönbaum and Sellers, 1966).

Some controversy has arisen as to the actual sites of NST when an animal is subjected to cold stress. Much of the initial work was done with the white rat and it is here that lack of inter-species extrapolation of various physiological phenomena should be handled with care.

Hibernating animals (Hayward et al., 1965) and other species including the human neonate (Dawkins and Scopes, 1965), demonstrate large amounts of brown adipose tissue (B.A.T.). Evidence for the contribution of this tissue as a body heat supply is readily acceptable due to the sympathetic nerve supply, the blood supply, the sensitivity of the tissue to cold and the metabolic rate of the tissue in the cold (Smith and Roberts, 1964; Donhoffer, Sárdy and Szegvari, 1964). The white rat maintains very little B.A.T. and it was finally substantiated that N.S.T. in this animal is partly a function of the non-contractile processes of skeletal muscle (Hayward, 1967). Measurement of cytochrome-oxidase activity of maximal metabolic capacity (Jansky, 1966) suggests that although the carcass incremental non-shivering heat is by far the largest, the effect of liver activity should not be overlooked.

Work done with cold-acclimated white rats (Himms-Hagen, 1965)



suggested that much of the non-shivering effect of increased metabolism was due to acceleration of the triglyceride cycle of the B.A.T. Cold acclimated rats showed a greater mobilization and lipogenic effect of B.A.T. during severe cold stress when compared with thermoneutral controls. This was not seen in white adipose tissue (Steiner et al., 1968).

Schonbaum et al., (1966) followed up this work by considering the possible controls of lipid utilization. They realized the control of beta-adrenergic receptors on lipid metabolism and blocked these receptors in rats acclimated at 40°C with propranolol in doses of 0.3 to 0.9 mg/kg intraperitoneally every 30 minutes. These anesthetized rats were placed in temperatures of 4°C and the effect of shivering was found to be greater compared to the non-treated trials. Thus, it was concluded that beta-adrenergic blockade did interfere with non-shivering thermogenesis in the rat.

Noradrenaline infusion has been shown to have calorogenic effects in rats (Hsieh, et al., 1957), kittens (Moore and Underwood, 1963) cats (Hemingway, 1964) dogs, and neonate rabbits (Heroux, 1967). Hsieh et al., (1966) demonstrated that although the metabolic rate of warm-acclimated rats increased slightly with noradrenaline infusion, similar infusion into cold-acclimated rats increased oxygen consumption from two to five fold depending on the dosage infused (these rats were acclimated to +5°C). The Canadian Cold Physiology Conference in 1967 suggested this procedure to demonstrate acclimation in animals. Noradrenaline infusions to warm-acclimated rats for 30 minutes has increased the basal (resting) metabolic rate 25 - 45%.

Increased metabolic rate in sheep has been shown to be highly





correlated with increasing heart rate during cold exposure (Webster, 1967). A relationship between the intensity of shivering in cold exposure and heart rate (Sykes and Slee, 1968) constitutes additional information to support these correlations. From the foregoing discussion it can be seen that during cold stress increases in both energy exchange and heart rate are mediated at least in part by the sympathetic nervous system. The present study was designed to demonstrate the extent of involvement of sympathetic control of energy metabolism and heart rate in the sheep.

#### E. Physiological Changes Associated with Feeding

The Agricultural Research Council's report on nutrient requirements of ruminants (1965) have suggested that the energy cost of grazing is too small to warrant any additional allowances when estimating the energy requirements of the animal at pasture. Any fundamental definition for the energy cost of feeding should include energy required for muscular jaw movements, increased gut and other organ movements and the processes of secretion and absorption. Grazing, of course, also involves increased movement of the animal in search and gathering of food. The energy cost of grazing must, however, include eating as one of the components of total energy need.

Graham (1964) estimated that the energy cost of eating cut herbage or grazing in a calorimeter was about 9 cal/min of eating/kg body weight for sheep. However, the time spent grazing the turf was considerably longer than the time spent eating an equivalent amount of cut herbage. He concluded that the energy cost of grazing was a function





of the time spent eating; a sheep which had grazed for ten hours would thus expend ten times the amount of energy compared to the sheep which had eaten for one hour.

Increases in energy expenditure were recorded 60% above resting levels during the first ten minutes of eating in tracheostomized sheep (Blaxter and Joyce, 1963). The increase in metabolic rate at the onset of eating was attributed to excitement experienced by the sheep during this time. Similar excitement was assumed to have occurred with the sheep Graham had used (Webster, 1966) so that those values of energy expenditure for the first hour could not necessarily be extrapolated for subsequent periods of eating time. Webster suggested that similar excitatory responsiveness to eating would not be exhibited by free ranging animals as they began to graze.

Webster (1967) measured the relationship between eating and metabolic rate of tracheostomised sheep. Oxygen consumption reached a maximal level during the first ten minute period of eating but began to decrease considerably before feeding was completed. After feeding (about 25 minutes) oxygen consumption values returned to levels comparable to those of pre-feeding. Heart rate did not attain a maximal peak until a fifteen minute period had elapsed. After eating, heart rate values did not return to the level of pre-feeding but were considerably greater for a period of at least 90 minutes. It was suggested that this initial metabolic response was a consequence of eating, per se, and not increased sympathetic activity due to excitement.

In a thermoneutral environment, grazing sheep demonstrate an



energy demand above basal levels which is related to standing, walking and eating. The energy demand which each warrants is determined by the availability of grasses to the animal. Consequently, a pasture which is quite productive and easily grazed would require less walking time and less searching (thus less eating time) to consume maintenance levels. Poorly developed pasture would force the sheep to walk farther and search for a longer period of time to achieve equivalent levels of feed. This would then result with a greater energy demand for grazing.

Heart rate has been used as an index of metabolic rate for man (Read, 1924; Malhotra, Gupta and Rai, 1963), cattle (Blaxter, 1943) and sheep (Blaxter, 1948; Webster, 1967) in various basal and active physiological conditions. Webster (1967) indicated that a close linear relationship existed between metabolic rate and heart rate when the stimulus for change was either cold exposure or eating. Since eating in the sheep is a form of physical activity, it is reasonable to suppose that the metabolic responses noted may be similar to those during muscular exercise.

The present experiments were designed to compared the metabolic and cardiovascular responses of sheep to the cold exposure and to eating while investigating the role of the sympathetic nervous system in both cases.



## II. GENERAL EXPERIMENTAL PROCEDURES

### Experiments at the University of Alberta

Experiments were designed to investigate the autonomic neural control of heart rate and energy metabolism in sheep when feeding and when exposed to acute cold stress. The technique of pharmacological blockade was used to inhibit sympathetic and parasympathetic control of these parameters. The experiments were conducted at the Environmental Laboratory at the University of Alberta Farm.

#### A. Animals and Rations

The five sheep used in the first experiment, which involved feeding trials and cold trials, were adult Lincoln males 60 to 100 kg in weight with fleeces up to 5 cm in depth. These animals were maintained in a thermostatic controlled thermoneutral environment (TN) at about +20°C (Blaxter, 1962) in individual feeding crates and fed 500 gm of alfalfa-brome hay two times per day.

The animals used in this project were accustomed to the procedures employed in these experiments. Heart rate measurements with various types of electrodes, and several metabolism measurements both with the mask and with the feeding hood had been carried out before the present series of experiments began. Several jugular catheterizations had also been performed prior to these trials. Consequently these animals were very familiar with the workers and equipment before any major data was acquired.

The twelve sheep used for the second experiment, a study in cold stress at summit metabolism were Suffolk males 60 to 80 kg which



had been on trial throughout the winter. They, thus, were also familiar with the workers and attachment of equipment. They had been maintained on good alfalfa-brome hay (offered at a level of two times maintenance) and were on the same feed during these trials in the spring (May). Their fleeces had been sheared one week before the experiment had started and were not clipped throughout the trials which lasted up to three weeks.

The pharmacological heart blockade trials with a propranolol-atropine infusion (Experiment 3) were performed with two Columbia-Lincoln lambs and with the same five Lincoln sheep of Experiment 1 after two of these animals had been thyro-parathyroidectomized for two months. These were maintained in the control room (+20°C) and fed the same alfalfa-brome hay ration. Acute experiments involving neural stimulation under anesthetic were conducted on several miscellaneous sheep including Suffolk ewes and two Columbia-Lincoln lambs of 20 kg weight.

#### B. Measurement of Energy Expenditure

Energy expenditure was determined from continuous measurement of respiratory exchange using open circuit respiration apparatus (Webster and Hicks, 1968a). During the periods of cold exposure the sheep wore a face mask which was ventilated at a fixed rate (Fig. 1) measured by a wet air flow meter<sup>1</sup>. During severe cold stress under which the heat production of a sheep is increased by nearly five-fold, the volume of air expired never exceeded 800 l/hr (Joyce and Blaxter, 1964). A ventilation rate of 2000 l/hr was used for the present

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<sup>1</sup>American Meter Co., Erie, Penna., U.S.A.





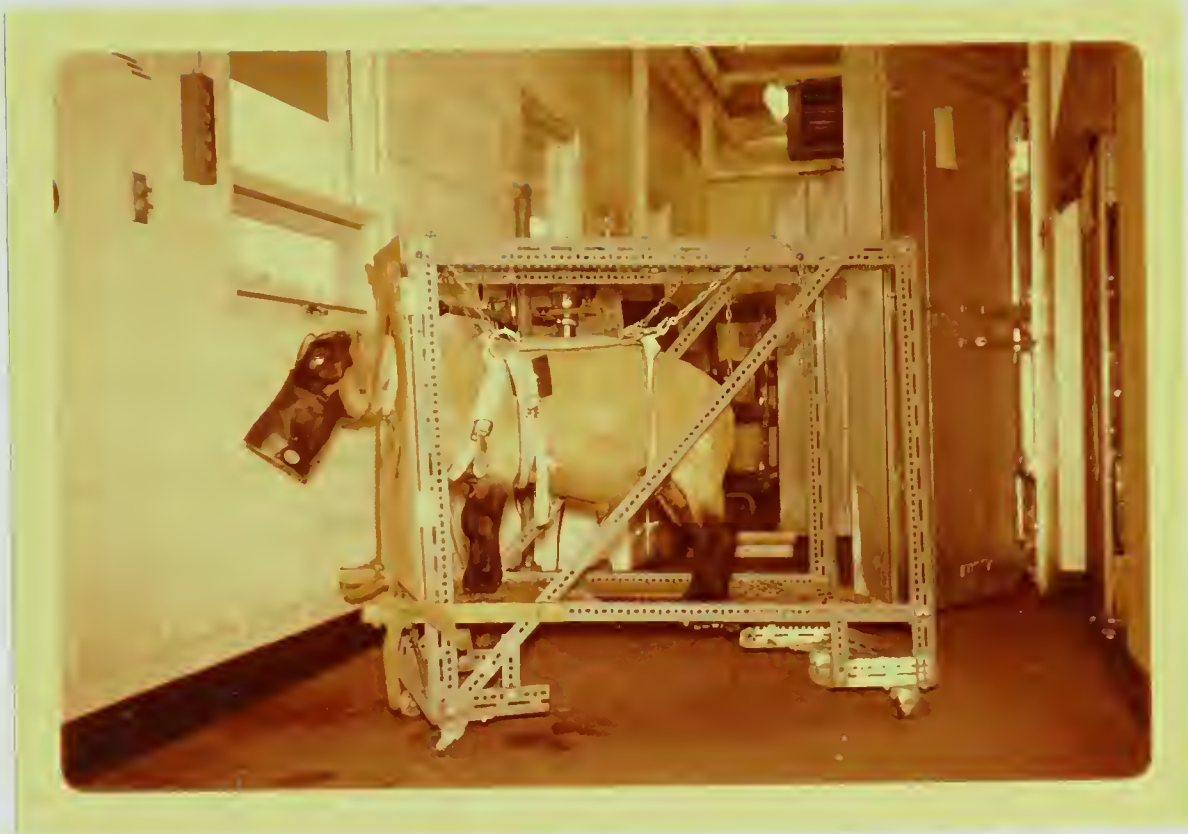


FIGURE 1 - View of sheep with respiratory mask in the movable crate.





FIGURE 2 - View of metabolism hood used for feeding trials.



experiments which was certain to collect all expired air from the sheep during all degrees of cold stress. The  $O_2$  and  $CO_2$  contents of an aliquot of the ventilating air stream were measured continuously using a Beckman F-3  $O_2$  analyzer and an IR-215  $CO_2$ <sup>2</sup> analyzer, respectively. In the feeding experiments, the head of the sheep was enclosed in a hood which was ventilated in a similar fashion. This hood was large enough to allow movement of the sheep's head within it and was enclosed about the sheep's neck by an air tight rubber collar and a strap. The front of the hood had a small window for observation and a small door which allowed feed to be placed before the animal and to be again removed (Fig. 2).

For the initial feeding and cold trial, the  $O_2$  and  $CO_2$  analyzers were both calibrated and operated with gases saturated with water vapor. This was done in order to obtain a rapid response to changes in  $O_2$  consumption and especially  $CO_2$  production. The absolute accuracy of the analyzers would have been greater, however, had the gases been dried (Webster and Hicks, 1968a). In the summit metabolism trials,  $O_2$  consumption only was measured. In this case, the air passing the gas analyzers was first dried with anhydrous calcium sulphate (Dri-rite<sup>®</sup>).

In no experiment, was account taken of  $CH_4$  production or N excretion in the estimation of heat production from measurement of respiratory exchange.

Energy expenditure (heat production,  $H_p$ , kcal/hr) was deduced from  $O_2$  consumption and  $CO_2$  production (l/hr) in the preliminary feeding trials and cold exposures according to the formula of Brouwer (1965):

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<sup>2</sup>Beckman Instruments, Inc., Fullerton, Calif., U.S.A.



$$H_p = O_2 \times 3.836 + CO_2 \times 1.200$$

For the calculation of energy expenditure in the summit metabolism studies the formula of Blaxter and Joyce (1963) was used:

$$H_p = O_2 \times 4.86$$

### C. Recording Heart Rates

Heart rates were usually obtained by radiotelemetry<sup>3</sup> or with the Sanborn physiological recorder<sup>4</sup>. Needle electrodes or surface electrodes were used throughout these trials. The radiotelemetry unit required only two electrodes and these were placed on the back above the second or third thoracic vertebra and on the left side behind the left leg (Fig. 3). These heart beats were counted as auditory signals from the radio receiver. The Sanborn physiological recorder preamplifier model 350-2800<sup>4</sup> indicated the electrocardiography (ECG) and heart rate measurements could be obtained by either counting the QRS peaks or integrated by use of a Sanborn Cardiotachometer preamplifier model 350-3400<sup>4</sup>. These measurements of ECG required three electrodes which were placed one on the back above the second or third thoracic vertebra and one on each side all in the same circumference just above each elbow.

Needle electrodes were placed just under the skin. Surface electrodes were applied to a well shaven skin surface with electrode jelly<sup>4</sup> as an aid to electrical transmission. The needle electrodes were tied to a belt around the animal with wire bag twisters (as used by food package industry) and the surface electrodes held in place with pads

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<sup>3</sup>Parks Electronics Ltd., Beaverton, Oregon, U.S.A.

<sup>4</sup>Hewlett-Packard (Canada) Ltd., Montreal, Quebec.







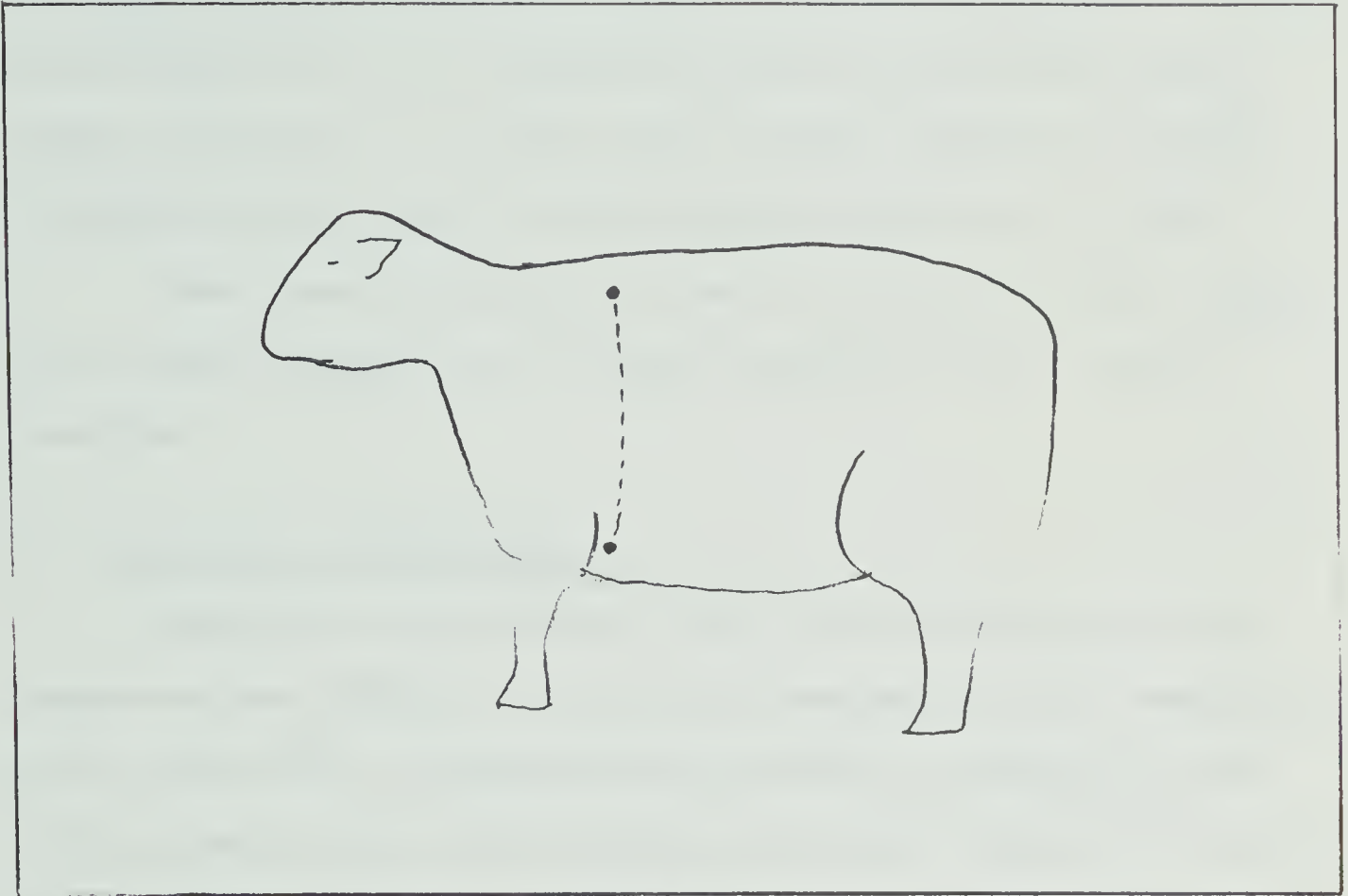


FIGURE 3 - Position of electrodes on the sheep for measure of heart rate by radiotelemetry.



about three inches square cut from rubber tubing or taped and glued to the fleece around the shaved area. It was found that either type of electrode gave good results for eating trials but the surface electrodes proved much better in cold exposure studies where shivering caused marked interference to the ECG signal and often loosened the needle electrodes allowing them to become detached from beneath the skin.

Heart rates were generally counted for each five minute period and 30 minute average heart rates were indicated unless otherwise specified.

#### D. Measurement of Temperatures

During the course of these trials, various estimates of body temperature were required. In the first experiment, rectal temperatures were recorded with a telethermometer<sup>5</sup> by means of a thermistor probe which was kept in the rectum throughout the trial. To ensure continued placement of the probe, it was held either with a tubing clamp which clipped the lead wire to the fleece or with a length of rubber tubing in the form of a loop tied to the harness of the crate. Ambient air temperatures were recorded with the same apparatus with the thermistor probe in the vicinity of the animal.

During the studies of cold stress at summit metabolism, temperatures were recorded on the Speedomax W recorder.<sup>6</sup> In total, twelve different temperature readings were possible and these included nine different skin temperatures, rectal temperature and two ambient air temperatures. Copper-constantan thermocouple junctions were attached

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<sup>5</sup>Yellow Springs Instrument Co., Yellow Springs, Ohio.

<sup>6</sup>Leeds and Northrup (Canada) Ltd., Toronto 15, Ontario.



to specific locations and connected to the recorder outside the cold exposure room.

#### E. Infusion of Drugs and Anaesthetic

Drugs and anaesthetic were administered through an intramedic polyethylene catheter<sup>7</sup> (PE -90/536") or (PE -190/536") which had been previously placed in either of the external jugular veins via a

bleeding needle (16-13 gauge). The catheter was threaded into the region of the vena cava proximal to the heart. This could be detected by having the catheter end into the left ventricle, indicated by pulsation of a small air bubble in the catheter at the syringe end, and then pulling about two or three inches of catheter out of the animal. The catheter was then filled with heparinized saline<sup>8</sup> (200 U.S.P. units of heparin per ml. of physiological saline) and shut off with a tap which was connected to the catheter by an 18 or 20 gauge needle (specific to catheter used). This tap was then tied to a collar about the animal's neck with bag twisters and was ready for use.

Drugs were infused into the animals by using a variable speed infusion pump (series 600 - 950 V)<sup>9</sup> which was kept on a moveable surgical cart for easy transport to and from the animal.

##### a) Drugs:

Propranolol (I.C.I.45,520, Inderal) was supplied by Ayerst Laboratories, Saint Laurent, Quebec courtesy of Dr. R.O. Davies. The

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<sup>7</sup>Clay-Adams Inc., New York, U.S.A.

<sup>8</sup>Riker Pharmaceutical Co. Ltd., Cooksville, Ontario.

<sup>9</sup>Harvard Apparatus Co. Ltd., Dover, Mass., U.S.A.



drug, in the powder form, was prepared freshly for each weekly set of trials by dissolving in physiological saline at a concentration of 2 mg/ml. Solubility was enhanced by heating the reagent bottle containing the saline and drug powder under warm tap water while shaking. Once in solution this drug did not tend to settle out.

Atropine solution was prepared with atropine sulphate and physiological saline at a concentration of 0.5 mg/ml. (The British Drug House (Canada) Ltd., Toronto, Ontario).

Isoproterenol solution was prepared from Isoprel injection U.S.P. (isoproterenol hydrochloride) in saline at a concentration of 10  $\mu$ g/ml

(Winthrop Laboratories, Division of Sterling Drug Ltd., Aurora, Ontario).

Noradrenaline (levophed) was prepared at a concentration of 10  $\mu$ g/ml, (Winthrop Laboratories, Division of Sterling Drug. Ltd., Aurora, Ontario).

Adrenaline (1-adrenaline) was supplied in the powder form (Eastman Organic Chemicals, Rochester, New York, U.S.A.) and was prepared by first adding a drop of concentrated sodium hydroxide to the solution and then adding saline to volume at a concentration of 10  $\mu$ g/ml (Merck Index, 1960).

Acetylcholine solution was prepared from acetylcholine bromide (Eastman Organic Chemicals, Rochester, New York, U.S.A.) by mixing with physiological saline to a concentration of 10 mg/ml.

b) Anaesthetic:

The anaesthetic, when required, was Nembutal (Pentobarbital





Sodium; (Abbott Laboratories Ltd., Montreal, Quebec), administered intravenously through the jugular catheter at an approximate dosage of 30 mg/kg given over a two to three minute period until the animal was in a surgically anaesthetized state. Subsequent amounts (usually about 2 mg/kg) were given whenever necessary. This additional volume was infused at a very slow rate (i.e., about 1-2 mg/kg/minute) to avoid any possible seizure due to sudden large amounts in the brain.

#### F. Statistical Analysis

Analysis of variance was carried out on most of the data obtained. Some of this was done with computer program BMD02V of the Computer Center at the University of Alberta. An indication of mean differences for treatments, times and animals was determined with "Duncan's New Multiple Range Test" (Steele and Torrie, 1960). The F.-values of 0.1% level were determined from the tables by Pearson and Hartley (1962).

Standard deviations for pooled data and simple regressions for heart rates and heat production were determined using APL computer programs MVSD and REG, respectively of the Computer Center (Smillie, 1968).



### III. Experiment 1.

#### Effects of Beta-adrenergic Blockade on the Heart Rate and Energy Expenditure of Sheep During Feeding and During Acute Cold Exposure

##### Objective

Energy, one of the most vital components of any ration, can be utilized by the animal in different ways and for different types of production depending on the requirement which has the greater preferential need. Basal requirements are usually met before productive requirements; consequently, information which can be used to determine specific energy expenditures above maintenance aid in possible prediction of production under specific conditions.

It has been shown that increases which occur in heat production of a sheep during eating and during cold exposure are associated in linear fashion with an increase in heart rate. Thus, it is a contention to use heart rate as an index of metabolic rate. Although, increased metabolism with cold exposure or eating result in similar increases in heart rate, some differences in controlling mechanisms have been suggested. This experiment was designed to study the effect of the sympathetic control on metabolism and heart rate in sheep when eating and when under cold stress using the beta-adrenergic blocking drug, propranolol.

##### Methods

Five adult Lincoln sheep in the weight range 60 to 100 kg were used as experimental animals. They were maintained on 1.0 kg of alfalfa-brome hay per day. Heat production (kcal/hr) was calculated



from respiratory exchange of  $O_2$  and  $CO_2$ . Heart rates were counted for each five minute period with average heart rate values recorded for each thirty minute period.

#### A. Cold Exposures

Six experiments were performed on each of the five sheep. Catheterization, when required, was carried out immediately before the animal was used in the trial. In four of the trials the sheep were first exposed to an air temperature of  $+4^{\circ}C$  for one hour. Respiratory exchange, heart rate and rectal temperature were recorded continuously. Propranolol was infused (i.v.) after 30 minutes at dose rates 0.25 ( $CP_1$ ), 0.5 ( $CP_2$ ) and 1.0 mg/kg body weight ( $CP_3$ ) over a period of 15 minutes. No propranolol was infused in the fourth experiment which served as a control (C.Con). At the end of the first hour, the sheep were transferred immediately to a refrigerated room maintained at a temperature of  $-30^{\circ}C$ . Measurements of respiratory exchange, heart rate and rectal temperature were resumed within 2 minutes and continued for a further 4 hours. This delay was due to the time required to move the animal from one room to the next room, connect the air line and ECG terminals and to an instrument-warming period for the radio transmitter. Heart rates which were recorded continuously on a tape recorder during some of these trials while the sheep was moved changed very little during this period.

In two of the trials, the sheep were first exposed to an air temperature of  $+20^{\circ}C$  (thermoneutral, Blaxter, 1962) for one hour and then transferred to a room temperature of  $+4^{\circ}C$  and measurements continued for





FIGURE 4 - The sheep is in the individual crate which is kept in the thermoneutral control room. Beside the crate is the pump used for infusing drugs.





a further 4 hours. Propranolol was infused as above at a rate of 0.5 mg/kg in one of these trials (TP<sub>2</sub>). The other trial served as a control (T.Con). Heart rate and energy expenditure were analyzed by analysis of variance.

#### B. Feeding Trials

Preliminary feeding trials involved the measurements of heart rate on the 5 sheep. All recordings were taken outside of the thermo-neutral control room area and the animals were watched through a window from the outer room. Feeding was done in the regular individual crates (Fig. 4) at an ambient temperature of +8°C after the animals had been fasted for two feeding periods. These sheep were usually fed 500 gm of hay in the morning at 7:00 A.M. and in the afternoon at 4:00 P.M. These trials were usually conducted in the afternoon about 20 hours after the previous feeding. Catheterization, when required, was usually done just before the infusion. Heart rate was recorded at five minute intervals, 30 minutes before feeding. In three trials, a loading dose of propranolol was infused at dose rates of 0.25, 0.5 and 1.0 mg/kg during the 15 minute period immediately prior to commencing measurements. Thereafter, maintaining doses of 0.25, 0.5 and 1.0 mg/kg were infused during a further period of 60 minutes. In the fifth experiment, saline was infused at a rate of 0.5 ml/kg over 15 minutes, a volume comparable to infusion of 1.0 mg/kg propranolol-saline solution. An untreated control trial was also included. The sheep were offered 1.0 kg hay 30 minutes after the loading dose of propranolol had been given. One hour later any uneaten food was removed



and weighed. Heart rate measurements were continued for a further 45 minutes. An injection of 0.1 mg/kg isoproterenol was administered to all animals receiving propranolol at the beginning and end of the experiment to test the effectiveness of beta adrenergic blockade.

The procedure for the second set of trials was similar except that the sheep were fed in the ventilated hood to permit determination of energy expenditure during eating. Duplicate trials were performed on each sheep which included two control and two propranolol infusions (0.5 mg/kg). Measurements were started 30 minutes before the feed was made available to these animals. During the last fifteen minutes of this period, propranolol (when applicable) was infused over a fifteen minute interval and then the animals were fed. One hour later the remaining feed was removed and measurements continued for a further hour.

#### C. Time Effect of Propranolol Blockade

Trials were conducted to determine the time interval for effective beta-adrenergic blockade of propranolol. Three Lincoln sheep were used which had previously been trained in cold and eating experiments. The trials were performed with these animals in their individual crates inside the control room. Heart rate was continuously recorded on the Sanborn recorder and five minute heart rate mean were calculated. An estimate of resting heart rate was obtained thirty minutes before infusion of any drugs and then isoproterenol (0.2  $\mu$ g/kg) was injected via a jugular catheter. Two such isoproterenol injections, spaced fifteen minutes apart, were given. Propranolol (0.5 mg/kg) was given as



a single injection fifteen minutes after the last isoproterenol injection. Beta-adrenergic blockade was challenged every thirty minutes thereafter for six hours with injections of isoproterenol.

## Results

### Effects of Propranolol on Isoproterenol Induced Tachycardia

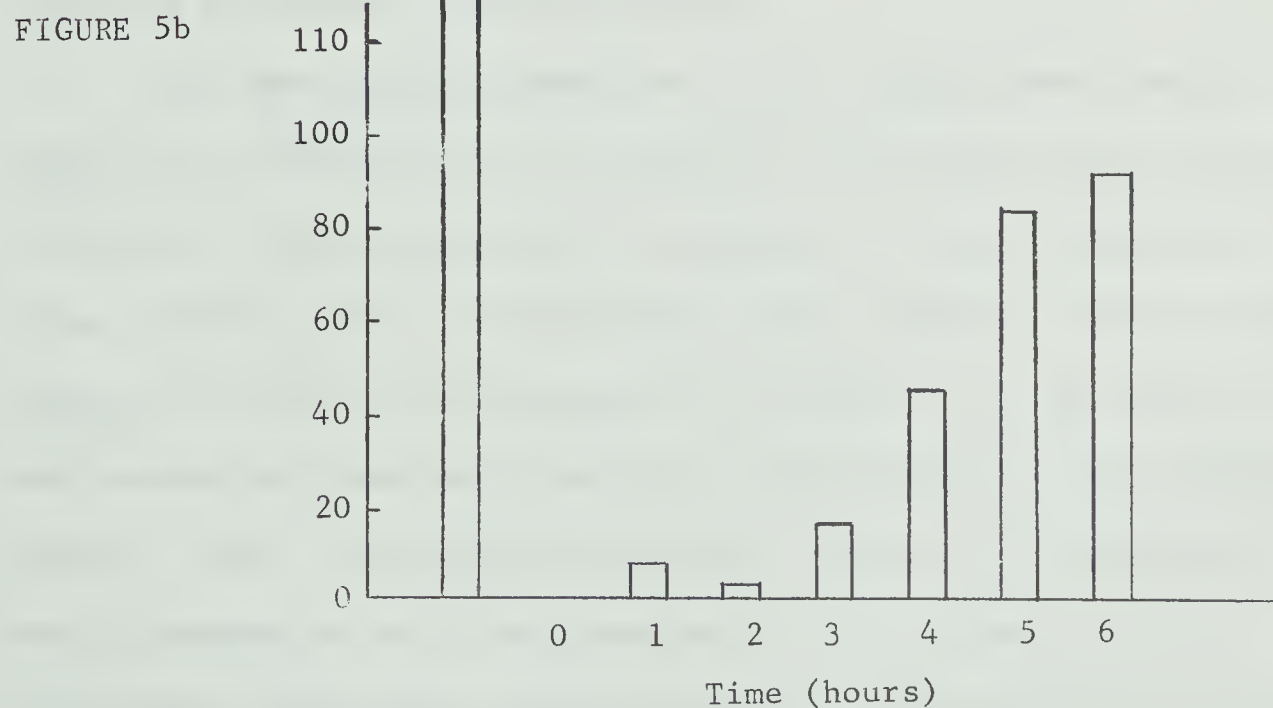
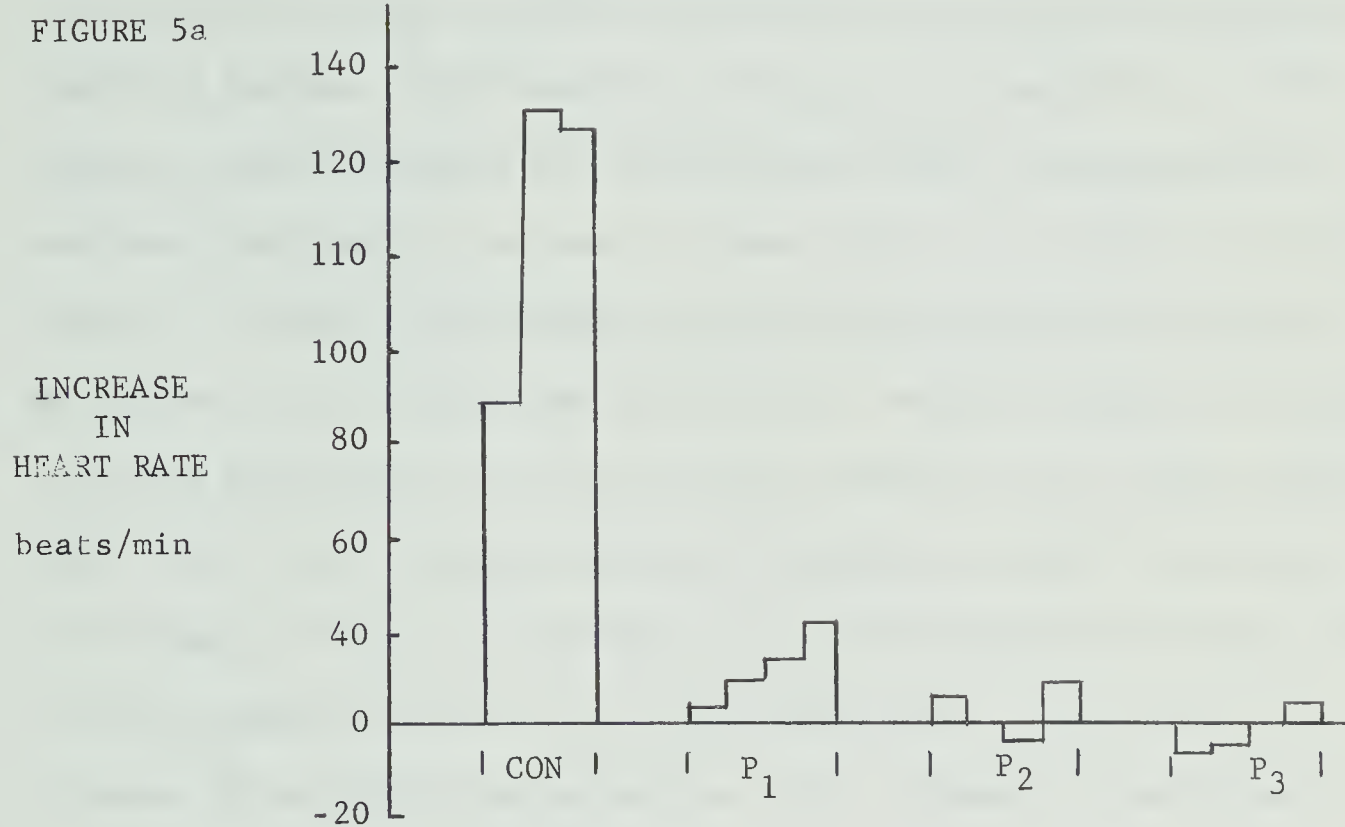
The effects of intravenous infusion of propranolol on the tachycardia resulting from intravenous injection of isoproterenol were studied in the sheep anaesthetized with nembutal. The mean heart rate of the sheep increased by 50 beats/min as a result of nembutal anaesthesia. Propranolol infusion at a rate of 0.5 or 1.0 mg/kg was sufficient to abolish the increase in heart rate following injection of isoproterenol at dose rates from 0.05 - 0.3  $\mu$ g/kg (Fig. 5a). Propranolol infusion at 0.25 mg/kg effectively blocked more than 90% of the tachycardia that resulted from injection of isoproterenol in the control experiments.

A single dose of 0.5 mg/kg propranolol in conscious sheep blocked more than 90% of the effects of repeated injection of 0.2  $\mu$ g/kg isoproterenol for at least 2 hours after injection (Fig. 5b). Further injections of isoproterenol at the end of the fourth and fifth hours after propranolol did produce an increase in heart rate of between 50-90 beats/min. These experiments indicate that the duration of effective beta-adrenergic blockade resulting from a single dose of 0.5 mg/kg propranolol to sheep was about 2 hours.

### Effects of Cold Exposure

Exposure of the untreated sheep to an air temperature of -30°C





The effect of i.v. propranolol on isoproterenol induced tachycardia.

a) Effect of propranolol at 0.25 (P<sub>1</sub>), 0.5 (P<sub>2</sub>) and 1.0 (P<sub>3</sub>) mg/kg on the increases induced in the heart rates of anaesthetized sheep by injection of 0.05, 0.1, 0.2 and 0.3  $\mu$ g/kg isoproterenol. No injection of 0.3  $\mu$ g/kg isoproterenol was made in the control experiment.

b) The effect of 0.5 mg/kg propranolol on the increase induced in the heart rate of conscious sheep by 0.2  $\mu$ g/kg isoproterenol at 1 hr intervals. Propranolol was infused after the first injection of isoproterenol.







(C.Con) produced an increase of about 60-70% in their heart rate and heat production (Fig. 6a). The mean heart rate of the untreated sheep reached a maximum of 130 beats/min, 90 min after exposure to  $-30^{\circ}\text{C}$ , falling slowly thereafter to 105 beats/min after 4 hours. Propranolol abolished this initial increase in heart rate at all levels of infusion. However, in these trials heart rate increased slowly to 95 beats/min by the end of the fourth hour of exposure. When the sheep were exposed to an air temperature of  $+4^{\circ}\text{C}$  following infusion of 0.5 mg/kg propranolol ( $\text{TP}_2$ ), heart rate remained relatively constant at about 70 beats/min for 150 min and then rose sharply to over 80 beats/min although no rise in heat production was noted in these experiments. Heart rate fell by about 10 beats/min during the four hour exposure of the sheep to  $+4^{\circ}\text{C}$  when propranolol was not infused.

The mean energy expenditure of the control sheep reached a maximum value of 180 kcal/hr or an increase of 70% after about 90 minutes of exposure to  $-30^{\circ}\text{C}$  and declined thereafter to reach a steady state at about 150-160 kcal/hr. This maximum value obtained for heat production during the initial period appeared, therefore, to be in excess of the heat production required to meet the thermal demand of the environment (Webster, 1966). The magnitude and time course of the increase in energy expenditure were very similar to those of heart rate. No significant differences existed between the values obtained for the energy expenditure of the sheep during exposure to  $-30^{\circ}\text{C}$  in the control experiments and following infusion of propranolol at 0.25 and 0.5 mg/kg. Propranolol infusion at 1.0 mg/kg, however, did reduce significantly ( $P < 0.001$ ) the magnitude of the steady state values for heat production



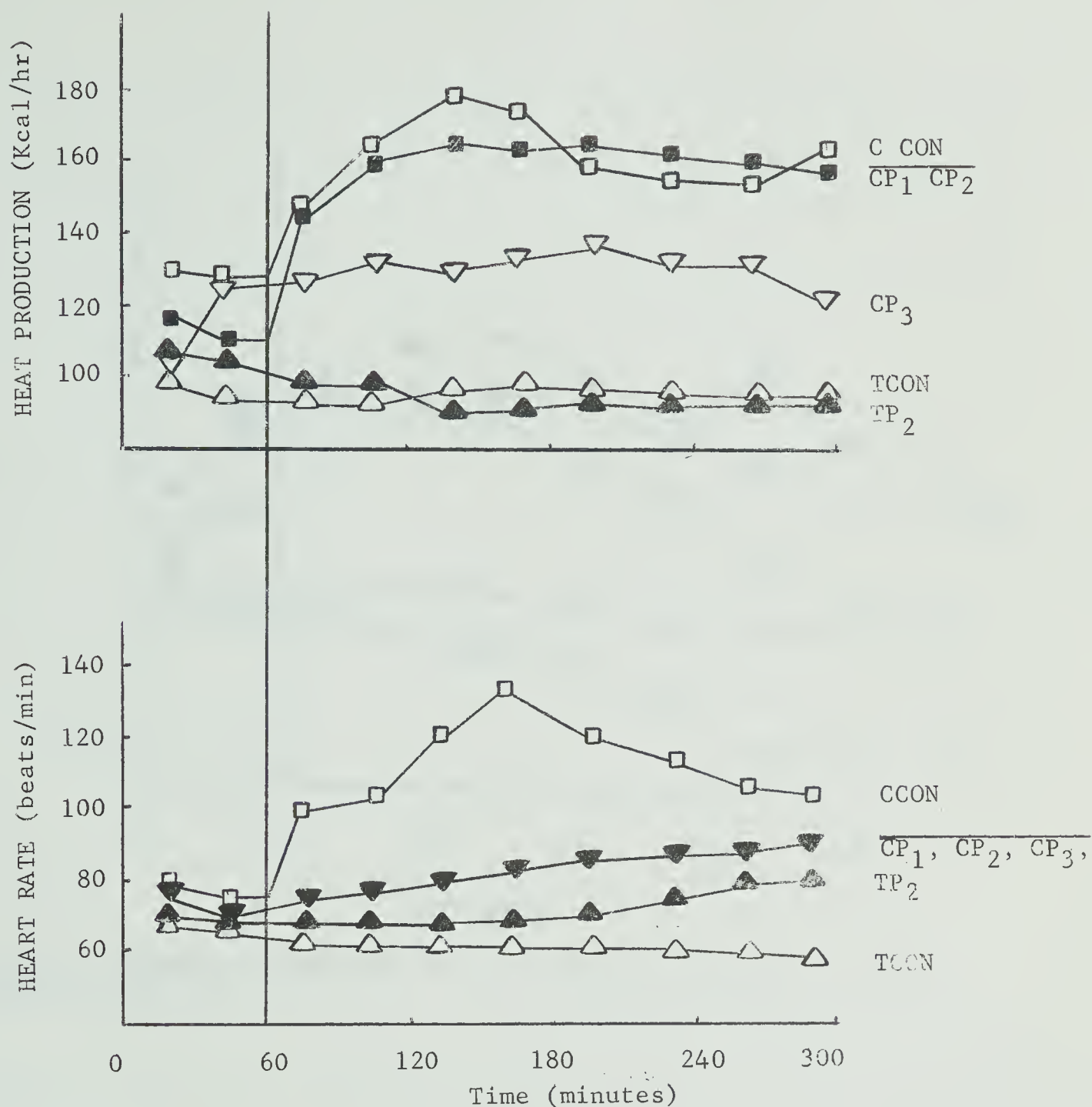


FIGURE 6a - Mean heart rates and energy expenditures of five sheep during exposure to cool ( $+4^{\circ}\text{C}$ ) and cold ( $-30^{\circ}\text{C}$ ) environments. In experiments C, Con.,  $CP_1$ ,  $CP_2$  and  $CP_3$  the sheep were first exposed to  $+4^{\circ}\text{C}$  for 1 hr and then to  $-30^{\circ}\text{C}$  for a further 4hr. In experiments  $CP_1$ ,  $CP_2$  and  $CP_3$ , propranolol was infused at 0.25, 0.5 and 1.0 mg/kg, respectively. In experiments T, CON and TP the sheep were first exposed to  $+20^{\circ}\text{C}$  for 1 hr and then to  $+4^{\circ}\text{C}$  for 4 hr. Propranolol (0.5 mg/kg) was infused in experiment  $TP_2$ .



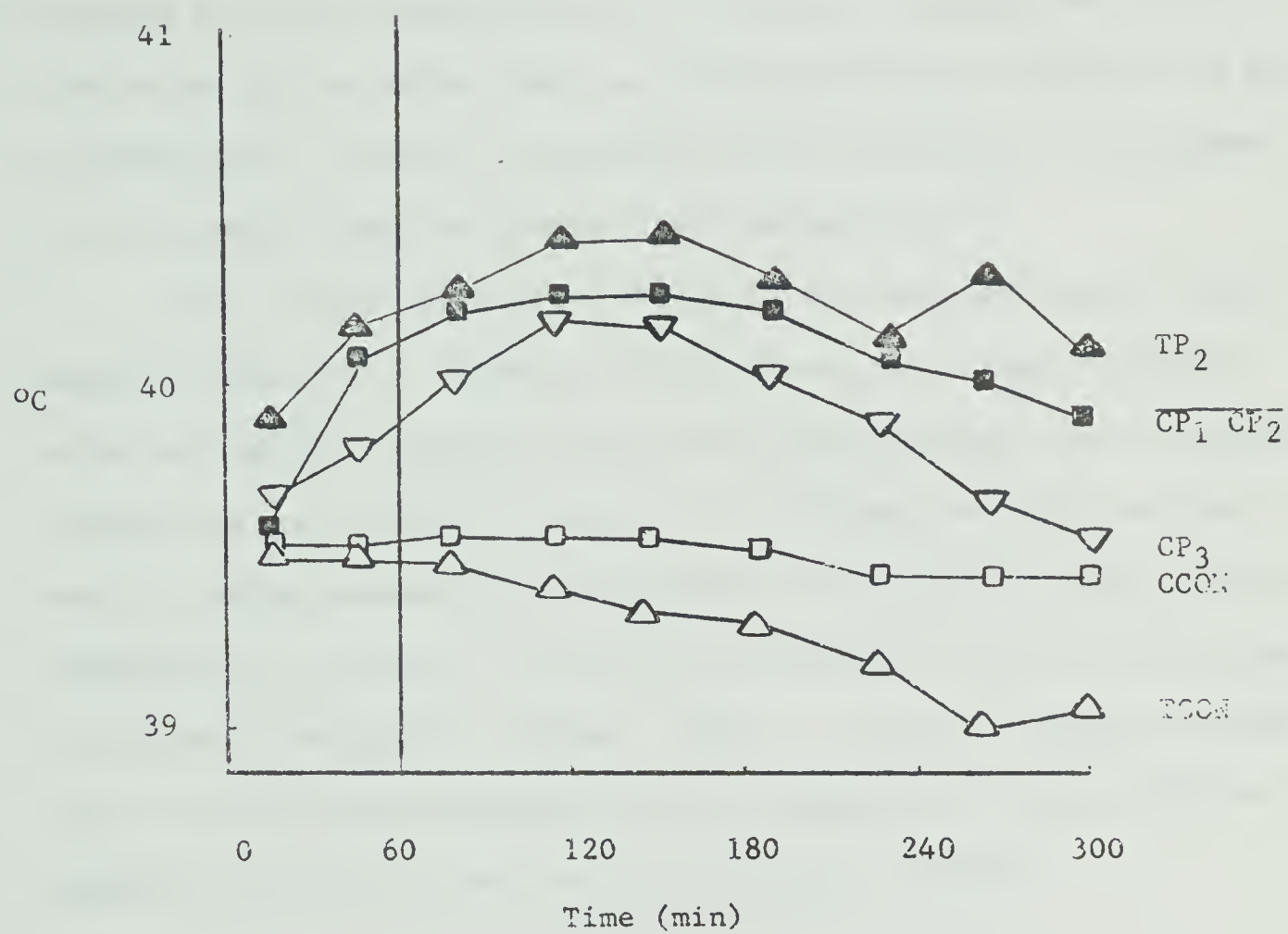


FIGURE 6b - Rectal temperatures of five sheep during the same time periods as Fig. 6a.



at  $-30^{\circ}\text{C}$  by about 14% and prevented the initial peak of heat production. Exposure to an air temperature of  $+4^{\circ}\text{C}$  did not increase the heat production of the untreated sheep, or of the sheep which received 0.5 mg/kg propranolol. Clearly, the sheep in these trials were not exposed to cold severe enough to elevate their metabolic rate.

Mean rectal temperature varied by less than  $1^{\circ}\text{C}$  in all experiments ( Figure 6b ). However, rectal temperature always increased after propranolol infusion; the increase being greatest when 0.5 mg/kg propranolol was followed by exposure of the sheep to  $+4^{\circ}\text{C}$ , and least when 1.0 mg/kg propranolol was followed by exposure to  $-30^{\circ}\text{C}$ . Rectal temperature is, however, an unreliable indicator of short term changes in deep body temperature, (Bligh, 1966). It was not possible, therefore, to relate these changes in rectal temperature to the different metabolic responses of the sheep to exposure to  $-30^{\circ}\text{C}$ .

#### Effects of Feeding

The results of the first series of feeding experiments, in which the sheep were fed in their metabolism cage ,are in Figure 7. The heart rate of the sheep in the control experiments increased rapidly at the onset of feeding, reaching a peak of 120 beats/min after about 20 minutes, then falling sharply after food was removed to values only slightly in excess of those recorded before the meal. The magnitude of the increase in heart rate noted during feeding was similar to that recorded by Young (1966) and Webster (1966).

Continuous infusion of propranolol produced a slight, though significant ( $P < 0.001$ ) reduction in the absolute values recorded for





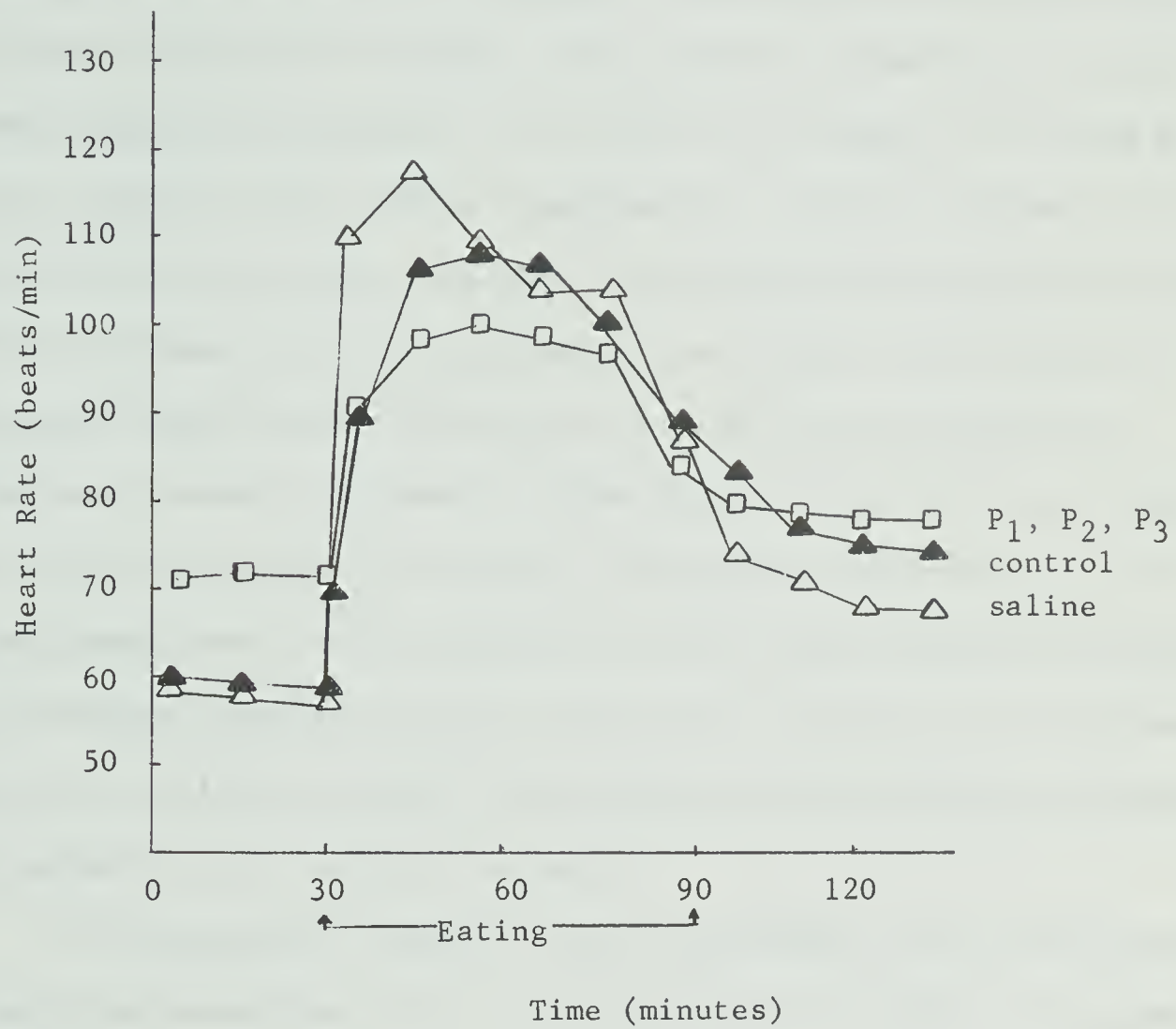


FIGURE 7 - Mean heart rates of five sheep before, during and after feeding in metabolism cages. P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> refer to propranolol infusion at 0.25, 0.5 and 1.0 mg/kg, respectively.



heart rate during the feeding period. The rate of cardioacceleration at the beginning of the meal and the heart rate after the meal were similar in treated and untreated animals. The increase in heart rate recorded during eating when propranolol was infused was, however, less than 60% of that observed in the control experiments. As Fig. 7 indicates, this resulted largely from the fact that heart rate before the meal increased in the experiments in which propranolol was infused. This point is discussed further below. There were no statistically significant differences between the absolute values obtained for heart rate at the three levels of propranolol infusion. Moreover, administration of 0.2  $\mu\text{g/kg}$  isoproterenol to the sheep at the end of the experiments produced no significant increase in their heart rates. As far as can be assessed, therefore, the effectiveness of beta-adrenergic blockade with propranolol was complete at all levels of infusion.

The results of a typical control experiment in the second series, in which the sheep were fed in a ventilated hood to permit measurement of their respiratory exchange, are illustrated in Fig. 8. The rate of  $\text{O}_2$  consumption increased sharply in this experiment to reach a peak within 10 minutes that was about twice that recorded before feeding. Production of  $\text{CO}_2$  increased at about the same rate but the peak was not achieved until about 30 min after the start of the meal by which time  $\text{CO}_2$  production was more than twice that recorded before the meal. Undoubtedly, a considerable proportion of the  $\text{CO}_2$  was belched from the rumen during eating (Blaxter and Joyce, 1963). However, the magnitude and rate of cardioacceleration that occurred during the meal were related much more closely to  $\text{CO}_2$  production than to  $\text{O}_2$  consumption. It is



reasonable to assume that the increase in heart rate was a direct result of an increase in arterial CO<sub>2</sub> tension resulting mainly from increased tissue metabolism but in part from absorption into the vascular system of CO<sub>2</sub> produced in the rumen.

The mean values obtained for heat production and heart rate during eating in the second series of experiments are summarized in Table 1. The heat production of the control sheep increased by 60-70% while they were eating. Infusion of 0.5 mg/kg propranolol did not significantly affect the magnitude of this increase. Heat production after the meal, however, was slightly greater ( $P < 0.001$ ) in the treated animals. Propranolol infusion in these experiments blocked about 45% of the increase in heart rate recorded during the meal but only reduced the absolute values recorded for heart rate during eating by about 15%.

Heart rate during eating in the treated and untreated animals were similar in both series of feeding trials although heart rate after the meal persisted at values well in excess of those recorded before the meal in the experiments carried out in the ventilated hood. However, the CO<sub>2</sub> content of sampled air leaving the hood rose at times to 2% during the course of the meal. This must have affected the normal cardioaccelerator response of the sheep to increased CO<sub>2</sub> production from tissue metabolism. The results of the first series of experiments, carried out in metabolism cages are, therefore, more likely to be truly representative of the normal changes that occur in the heart rate of sheep in association with eating.



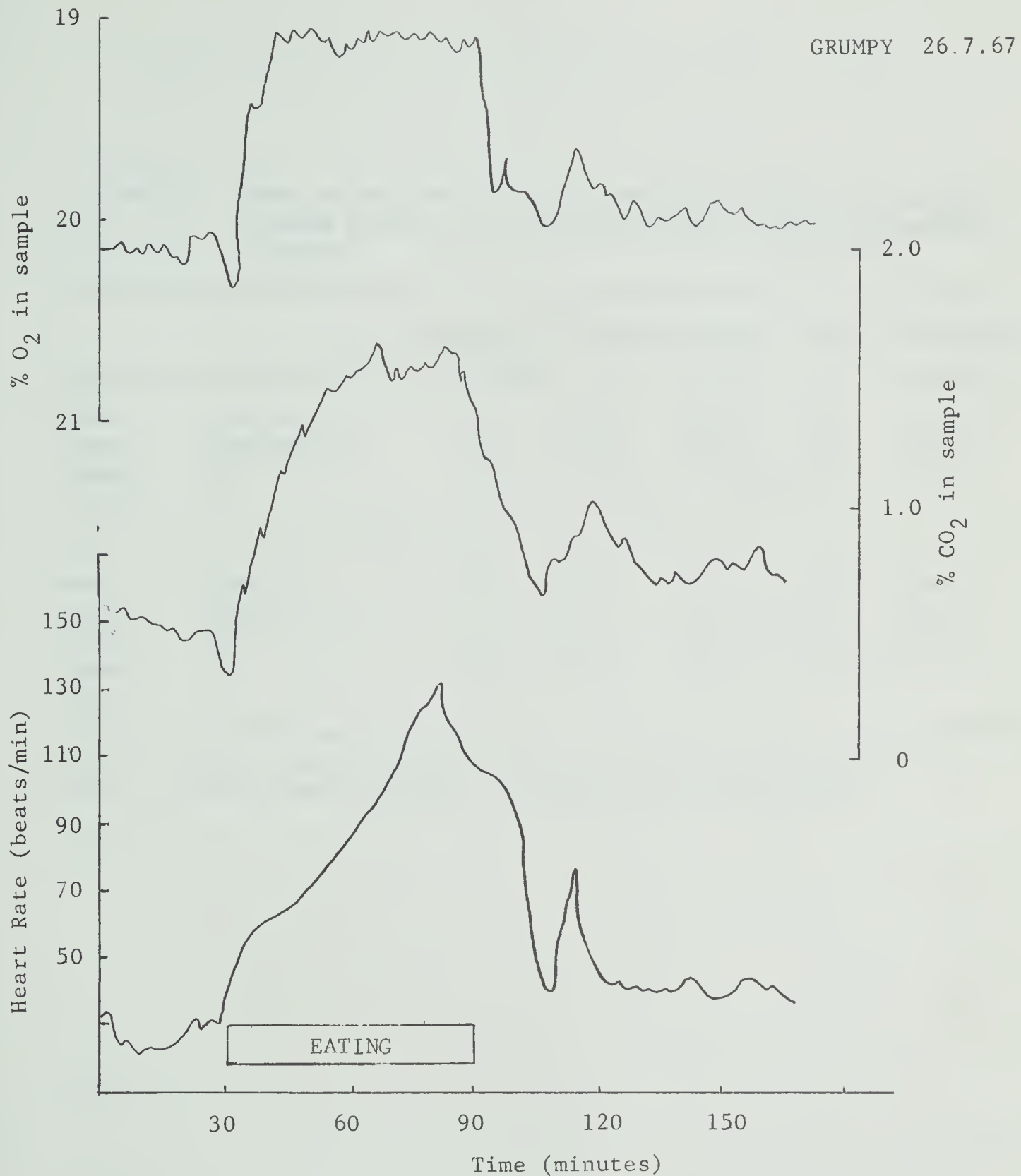


FIGURE 3 - Individual record of the O<sub>2</sub> consumption, CO<sub>2</sub> production and heart rate of sheep Grumpy during a feeding experiment in which no infusion was administered.





TABLE 1. Mean Values for Heart Rate and Heat Production of Five Sheep During 30 min Periods Before, During and After Eating

		Before feeding	During feeding		After feeding	
			1	2	1	2
Heart rate (beats/ min)	Control	57.3	91.4	118.0	95.1	82.3
	Propranolol	60.3	81.0	96.0	90.8	84.7
	$\bar{S}\bar{x}^*$	-----	-----	3.68	-----	-----
	p	N.S.	0.05	0.01	N.S.	N.S.
Heat production (kcal/ hr)	Control	94.0	157.1	167.1	113.4	122.7
	Propranolol	104.2	169.6	175.0	133.4	129.8
	$\bar{S}\bar{x}^*$	-----	-----	5.56	-----	-----
	p	N.S.	N.S.	N.S.	0.05	N.S.

\* $\bar{S}\bar{x}$  =  $\sqrt{\text{Error mean square/no. of replicates in each mean.}}$



### Effect of Propranolol on Resting Heart Rate

The mean heart rate of the sheep following propranolol infusion was greater than that recorded in the control experiments during the initial period before the stimuli of cold or feeding were applied. While the sheep undoubtedly were handled to a greater extent immediately prior to the experiments in which propranolol was infused, it is unlikely that this was the sole cause of the increase in heart rate. Infusions of saline, carried out in an identical fashion to the propranolol infusions, did not significantly alter heart rate from the values obtained in the control experiments in which no infusions were given (Table 1). Moreover, heart rate increased steadily during the four hour exposure of the sheep to a thermoneutral environment following infusion of 0.5 mg mg/kg propranolol (Fig. 6). When no infusion was given heart rate fell slightly. It appears, therefore, that the resting heart rate of the sheep in these experiments increased slightly as a direct result of propranolol infusion. This contrasts with results for dogs (Cronin, 1967) and man (Epstein et al., 1965) in which resting heart rate fell after propranolol infusion.

### Discussion

The increase that occurs in the heart rate and heat production of sheep during cold exposure and during eating has been attributed to the increased amount of energy expended in shivering, and in ingesting food, respectively (Blaxter, 1967). If both of these are considered primarily as muscular activities, then it would be reasonable to expect that the effects of beta-adrenergic blockade would be similar in the



two situations. The results of the experiments reveal, however, that considerable differences existed between the effects of beta-adrenergic blockade on the heart rate and heat production of sheep during feeding and during cold exposure.

Cronin (1967) reported that propranolol blocked about 50% of the absolute increase that occurred in the heart rate of dogs during exercise on a treadmill. The rate of cardioacceleration in response to sudden exercise was, however, very rapid in both cases; achieving 90% of the steady state response in less than 10 seconds. In the present experiment, the rate of cardioacceleration of normal sheep in response to a sudden cold stimulus closely followed the rate of increase of their heat production so that within 100 minutes of cold exposure heart rate was over 130 beats/min. The rate of cardioacceleration following propranolol infusion was very much reduced, so that after 100 min of cold exposure heart rate was only 84 beats/min. Moreover, some of the increase that occurred in heart rate during the third and fourth hours of cold exposure would appear to have been due in part to a wearing off of beta blockade. The increase that occurred in the heart rate of sheep during the third and fourth hours of exposure to a thermoneutral environment after propranolol infusion lends support to this conclusion. Beta-adrenergic blockade, then, completely abolished the initial cardioacceleration exhibited by sheep on exposure to cold although their metabolic rate was increased by about 80%.

The effect of feeding was to produce an increase in metabolic rate of comparable magnitude, which was not affected by propranolol



infusion. In these experiments, propranolol infusion had no effect on the initial rate of cardioacceleration at the beginning of the meal and only reduced mean heart rate during feeding from 105 to 93 beats/min. The reduction in heart rate was the same at all levels of propranolol infusion. This strongly suggests that the degree of beta-adrenergic blockade was complete in all cases. Clearly, therefore, a considerable degree of cardiac adaptation to the increased energy expenditure noted during feeding in the sheep can occur in the absence of sympathetic induced cardioacceleration.

This suggests that the stimulus to cardioacceleration during eating, but not during acute cold exposure, in the sheep, is comparable to the stimulus noted during running exercise in dogs (Cronin, 1967) and man (Epstein et al., 1965; Cumming and Carr, 1966), which appears to be to a considerable extent independent of the sympathetic nervous system. Donald and Samueloff (1966) in an elegant series of experiments showed that the denervated heart of the dog still increased in rate in response to exercise after blockade of blood borne sympathetic transmitter substances. They concluded that there was an intrinsic mechanism in the dog's heart that brought about cardioacceleration in proportion to the amount of work performed. The present results suggest that a similar mechanism may exist in the sheep.

Propranolol infusion at a rate of 1.0 mg/kg significantly reduced the magnitude of the increase in energy expenditure that occurred in response to cold, particularly the initial rise in heat production that appeared to overshoot the thermal demand of the environment. Heim and Hull (1966) reported that they were able to abolish the calorogenic







response to cold in new born rabbits with propranolol at a dose rate of 5 mg/kg, although 1 mg/kg propranolol had no effect. They concluded that the increased calorogenesis in new born rabbits exposed to cold occurred primarily in brown adipose tissue, and could be blocked by large doses of propranolol. The metabolic body size (weight  $\text{kg}^{0.75}$ ) of a 50 kg sheep is about 200 times greater than that of a 50 g new born rabbit. The absolute dose of propranolol administered to a 50 kg sheep at a rate of 1 mg/kg is exactly 200 times that administered to a 50 g rabbit at a dose rate of 5 mg/kg. The maximum dose of propranolol administered to the sheep in these experiments was thus comparable to that administered to new born rabbits by Heim and Hull. It is reasonable to conclude, therefore, that most of the increase in heart rate that occurs when sheep are exposed to cold, and some of the increase in energy expenditure, in particular the early metabolic overshoot, are initiated and mediated via the sympathetic system. The fact that the mechanism is sensitive to beta-adrenergic blockade suggests that it is not entirely dependent on the increased muscular activity of shivering. However, the exact sites and mechanisms of increased calorogenesis in the sheep in response to cold remain obscure.

The fact that the increased energy expenditure of the sheep during feeding was not affected by propranolol at any concentration strongly suggests that none of this increase can be attributed to sympathetic activity. This increase can be considered therefore, as an inevitable consequence of the work involved in the exercise of eating and not due to any excitement that the sheep might have experienced when receiving meals at regular hours in their metabolism



TABLE 2. Estimates of the Energy Cost of Different Activities for Sheep

Activity		Energy cost of activity cal/kg body weight per min		Source of data
Standing		1.96	(1.55-2.38)	Webster and Valks (1966)
Eating forage	1.	9.0	(4.0-16.3)	Graham (1964)
	2.	22 <sup>+</sup>	(22-23)	Webster (1966a)
	3.	13.8	(11.7-15.5)	Present experiments
Ruminating		4.0	(1.3-8.7)	Graham (1964)
Walking at 1.8 mph		33	(+4.8)	Clapperton (1964)

<sup>+</sup>Estimated from continuous measurement of heart rate during exposure to cold environments.



cages.

The act of eating forages, then, inevitably induces a considerable increase in the energy expenditure of sheep that does not persist into the post prandial period and is, therefore, not associated with the processes of digestion and metabolism of the ingesta; functions which are generally considered to constitute the heat increment of feeding (Blaxter, 1967). The true energy cost of eating was calculated from the present experiments to be 13.8 cal/kg body weight per min spent eating (Table 2). Previous estimates of the energy cost of different activities for sheep are also shown in Table 2.

It is clear from this table that the energy cost to sheep of eating forages is very considerable, about six times greater than the energy cost of standing (Webster and Valks, 1966) and almost half as great as the energy cost of walking at a normal pace of just less than 2 mph (Clapperton, 1964). The energy cost of eating forages is also about three times greater than that of rumination (Graham, 1964) which can be considered to be a comparable activity involving chewing and ensalivation of food material. Table 3 represents a comparison of heart rate and rumen and reticular motility before, during and after eating in one sheep. Gut motility was measured with pressure transducers through a rumen fistula. Upon eating rumen motility increased three fold and reticular motility increased two fold. Heart rate increased 20 beats/min. Although similar movement would be expected during rumination, a large amount of this increased metabolic requirement of eating in sheep may be due partly to this increased gut movement. Further study is required to explain this phenomenon. The



TABLE 3. Reticulum-Rumen Motility and Heart Rate during Eating

	Time (min)	Heart rate beats/min	Rumen motility*	Reticular motility*
Prefeeding	15	62	10	7
	30	54	8	8
	45	54	9	6
	60	53	11	8
Feeding	15	80	33	20
	30	72	32	23
Postfeeding	15	56	14	11
	30	52	14	9
	45	56	10	10

\*Contractions/min.

A comparison of heart rate and rumen and rumen-reticular motility before, during and after eating.





practical implications of the high energy cost of feeding are, however, quite clear. A sheep at pasture, which may have to graze ten hours a day to achieve sufficient food intake for maintenance and production (Arnold, 1962) may expend up to 300 kcal a day to elevate its metabolic rate by about 20% simply as a result of the act of grazing. This does not include the energy cost of standing to graze and walking to graze.

In 1965 the A.R.C. committee on the nutrient requirements of ruminants (A.R.C., 1965) concluded that the energy cost of grazing was small enough to be neglected when calculating the energy requirements of cattle and sheep. The present experiments clearly indicate that this conclusion invites reconsideration.

#### Summary

1. Resting heart rate in sheep increased following propranolol blockade by about 20 beats/min. This contrasts with a drop in heart rate recorded in man and dogs after treatment with propranolol.

2. Feeding tachycardia in sheep is not effectively inhibited by beta-adrenergic blockade.

3. Propranolol infusion at levels of 0.25, 0.5 and 1.0 mg/kg effectively blocked beta-adrenergic receptors of the heart in sheep for a period of at least two hours.

4. Increased metabolic response due to cold exposure was blocked only with doses of propranolol of 1.0 mg/kg. Other concentrations used (0.25 and 0.5 mg/kg) were not effective in controlling energy metabolism.



#### IV. Experiment 2

##### The Effects of Beta-adrenergic Blockade in Sheep at Summit Metabolism

#### Objective

It was shown in the previous experiment that beta-adrenergic blockade with propranolol (1.0 mg/kg) would block the "metabolic overshoot" demonstrated by untreated sheep when subjected to a sudden cold exposure. The present trials were designed to determine the effect of beta-adrenergic blockade in sheep which were subjected to cold to provide maximal or near maximal stress.

#### Methods

This experiment involved twelve Suffolk sheep, divided into three groups of four animals which had experienced different environmental conditions throughout the preceeding winter from October to May. The control animals were kept in a thermostatically controlled room at +8C<sup>0</sup>. The outdoor sheep were kept outside throughout this period in an open lot with no overhead shelter but with some wind shelter from the north provided by the laboratory building. The indoor sheep were kept at a temperature calculated to provide an equivalent degree of cold stress to that experienced by the outdoor group. This effective air temperature was calculated from average air temperature, windspeed and solar radiation for a week long interval. The indoor sheep were exposed to this during the following week. The animals were maintained on alfalfa-brome hay throughout the experimental time. The main part of this work has been reported briefly elsewhere (Webster and Hicks, 1968b).



The sheep were sheared in late April and this experiment ran from this time until the third week in May. The experiment included a control (drug-free) and a propranolol (1.0 mg/kg, i.v.) infusion for each animal. Propranolol was infused prior to the onset of cold exposure. The sheep were exposed to a nominal air temperature of  $-30^{\circ}\text{C}$  for three hours.

Measurements of heart rate and rectal and skin temperatures could be started two minutes after entry into the cold. Heart rates (Section II) were determined every five minutes and mean values for every thirty minutes were analyzed. Skin temperatures were determined by placing surface thermocouples at designated locations on the sheep which included the shoulder, rib and flank areas on both sides, on an ear and the front and hind feet (on the hock) (Fig. 9). The required areas of the skin were shaved and thermocouple ends were fastened to circular patches of black polyethylene sheeting about 10 mm. in diameter with surgical tape and paper glue. A twelve channel temperature recorder plotted the temperatures and an estimate of mean skin temperature was calculated with weighted values of 10% for the extremities (legs and ears) and 90% for the trunk (Blaxter, et al., 1959).

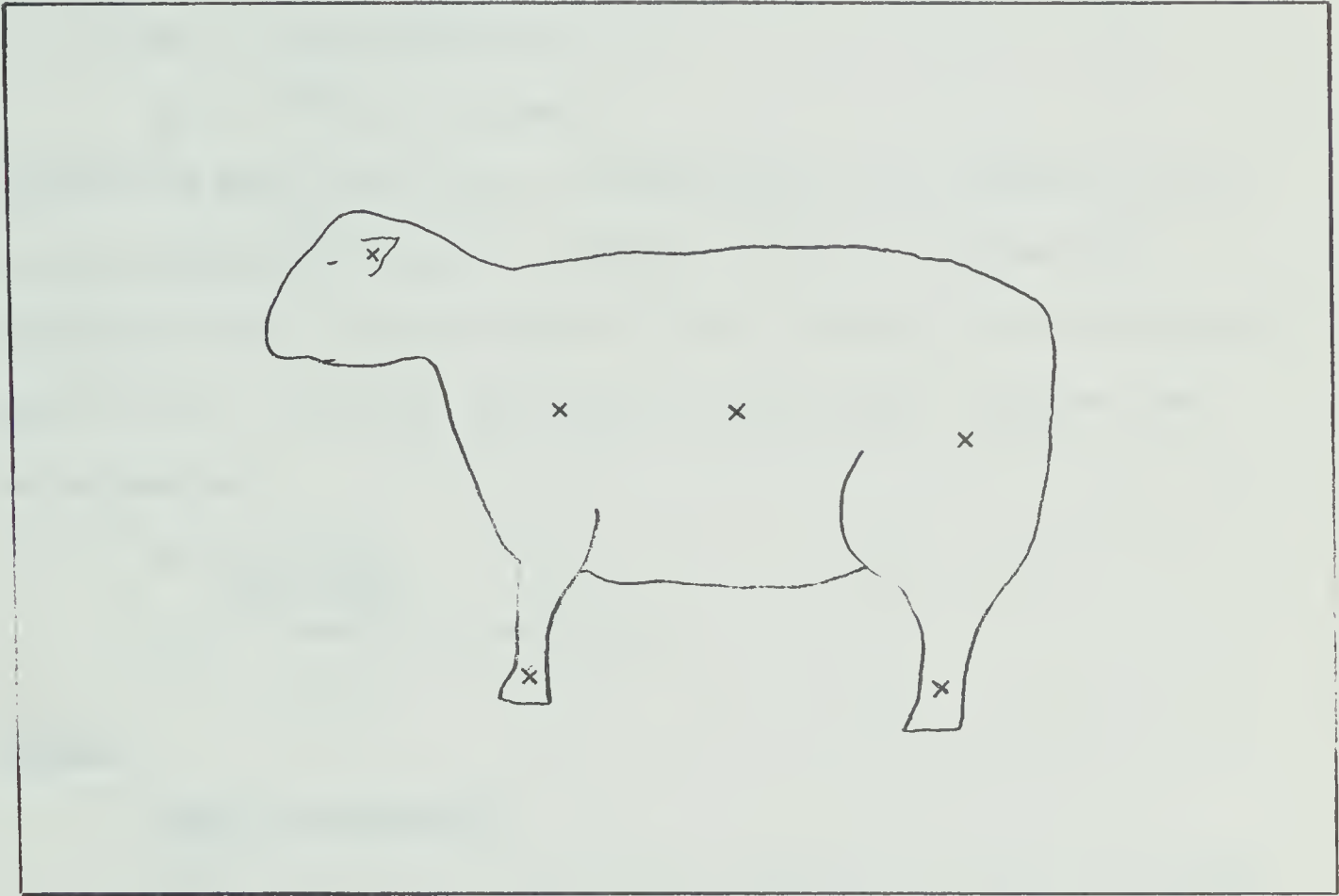
Rectal temperatures were also measured using a thermocouple probe and plotted continuously on the recorder.

Heat production was calculated as:

$$H_p = O_2 \times 4.68 \text{ kcal/hr (Blaxter and Joyce, 1963)}$$

Comparisons of mean temperature differentials ( $T_R - T_S$ ) were calculated where  $T_R$  is the rectal temperature ( $^{\circ}\text{C}$ ) and  $T_S$  is the





2. Attachment of thermocouples for skin temperature measurements. The position of the three thermocouples on the shoulder, rib and flank were duplicated on the right side as well.





mean skin temperature ( $^{\circ}\text{C}$ ).

Tissue insulation was measured as:

$$I_T = \frac{T_R - T_S}{H_p}$$

$$H_p \dots \text{Mcal/m}^2 \cdot 24 \text{ hr.}$$

$$I_T \dots ^{\circ}\text{C} \cdot \text{m}^2 \cdot 24 \text{ hr/Mcal}$$

Evaporative heat loss could not be measured in this experiment, but previous experiments (Joyce and Blaxter, 1964), have shown that evaporative heat loss from sheep in cold conditions to be 0.25 to 0.30 Mcal/m<sup>2</sup>·24 hr. External insulation for the present experiment was calculated as:

$$I_E = \frac{T_A - T_S}{H_p - 0.30}$$

$$T_A \dots \text{mean air temperature.}$$

## Results

### Control experiments

Table 4 shows the mean results obtained for rectal, skin and air temperatures, rectal-skin temperature differential, metabolic rate and heart rate during acute cold exposure ( $-30^{\circ}\text{C}$ ). Mean air temperature for all trials did not vary more than  $2.5^{\circ}\text{C}$ . No significant difference was evident between mean rectal temperatures in the non-propranolol (drug-free) trials. After the sheep has been moved to the cold temperature ( $-30^{\circ}\text{C}$ ) rectal temperatures increased over the first thirty to sixty minutes to values not more than  $0.5^{\circ}\text{C}$  above the pre-exposure level and then began to decrease steadily to values not more than  $1^{\circ}\text{C}$  below pre-exposure levels (Fig.10 ). The rectal temperature of the drug-free controls increased from  $39.2$  to a maximum of  $39.7^{\circ}\text{C}$  during the first hour of exposure and then began



TABLE 4a. Acute Cold Stress

		Mean(1) $T_R^{\circ C}$	Mean(2) $T_S^{\circ C}$	Mean(3) $T_R - T_S^{\circ C}$	Mean(4) $T_A^{\circ C}$	Mean(5) H.R.*
Without	Control	39.4	13.5	25.9	-27.1	192
Propranolol	Outdoor	39.4	13.0	26.4	-28.7	150
	Indoor	39.3	10.6	28.7	-28.5	214
Propranolol	Control	39.5	12.8	26.7	-27.1	118
	Outdoor	39.6	14.0	25.6	-28.8	98
	Indoor	40.0	12.8	27.2	-26.7	112

TABLE 4b.

		HEAT PRODUCTION (6)			
		Mcal/m <sup>2</sup> .24hr	Kcal/kg <sup>0.75</sup> .24 hr	I <sub>T</sub> **	I <sub>E</sub> **
Without	Control	5.021	313	5.2	8.6
Propranolol	Outdoor	3.500	219	7.5	13.0
	Indoor	6.148	392	4.7	6.7
Propranolol	Control	4.628	293	5.8	9.3
	Outdoor	3.528	222	7.3	13.4
	Indoor	5.377	347	5.1	7.9

For significance see the following:

- (1) Appendix 11, (2) Appendix 12 (3) Appendix 13  
 (4) Appendix 14, (5) Appendix 9 (6) Appendix 10

\* Heart Rate beats/min

\*\*  $^{\circ C} \cdot m^2 \cdot 24 \text{ hr} / \text{Mcal}$ .



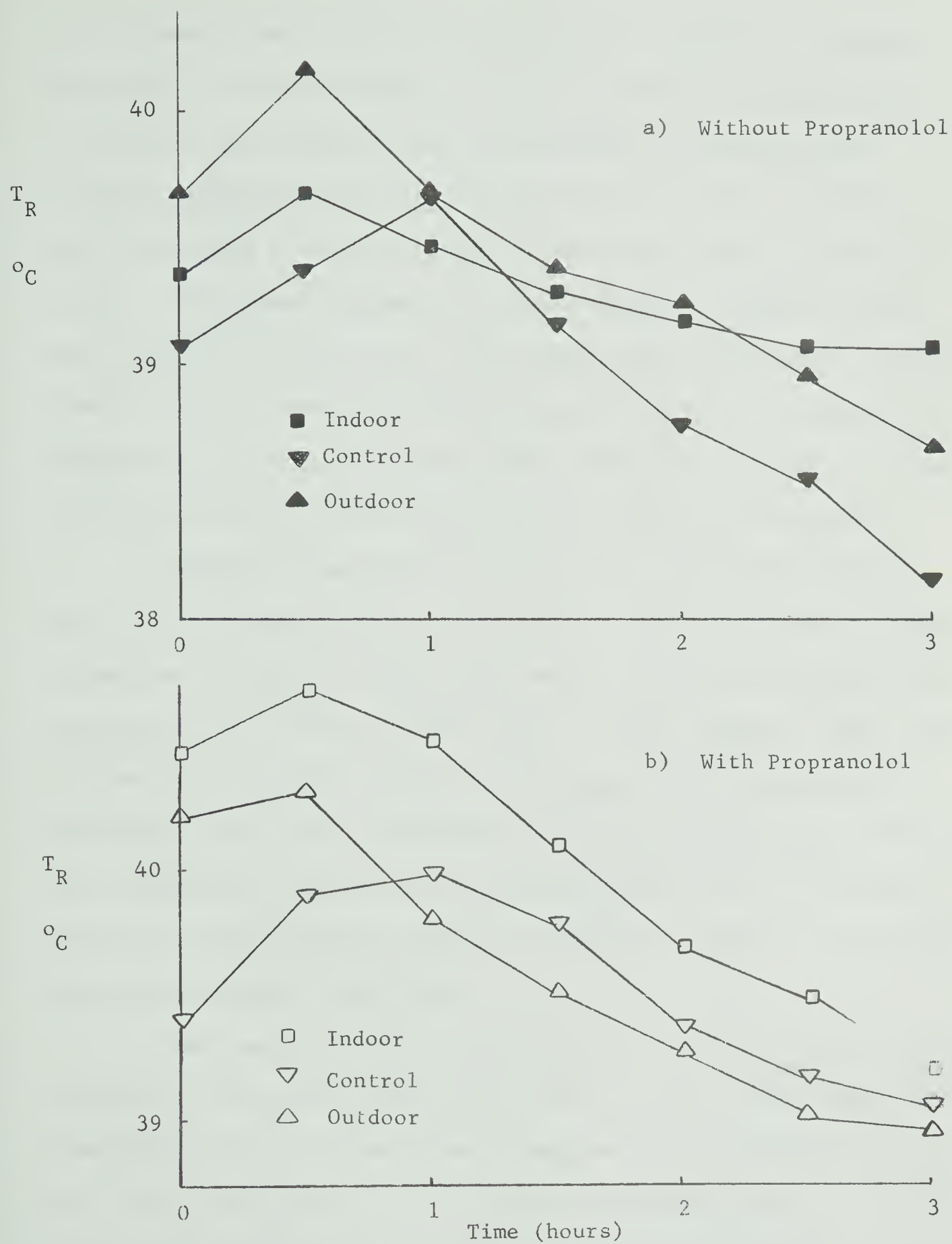


FIGURE 10 - Rectal temperatures of control, indoor and outdoor groups:  
a) Without and b) With Propranolol.



to decrease steadily to  $38.2^{\circ}\text{C}$  by the end of the hour of exposure (Fig. 10a). Similar patterns of rectal temperature response were followed by the drug-free outdoor sheep which increased rectal temperature from  $39.7^{\circ}\text{C}$  to  $40.2^{\circ}\text{C}$  over the first thirty minutes and then showed a decrease to  $38.7^{\circ}\text{C}$  three hours after initial cold stress. The decreasing rates of rectal temperature were  $0.50^{\circ}\text{C/hr}$  and  $0.55^{\circ}\text{C/hr}$  for the control and outdoor sheep respectively. Rectal temperature increased only  $0.3^{\circ}\text{C}$  during the first thirty minutes of exposure for the drug-free inside sheep and then decreased at a rate of  $0.2^{\circ}\text{C/hr}$  for the remaining two and one half hours of exposure.

Mean skin temperature for the entire cold exposure period was  $13.5$ ,  $13.0$  and  $10.6^{\circ}\text{C}$  for the control, outdoor and indoor groups, respectively (Table 4). Mean skin temperature has been suggested as an index for the degree of cold stress in sheep (Webster, 1966). It is reasonable to assume that the indoor group was losing slightly more heat to the cold environments than the other two groups. Mean skin temperature differentials ( $T_R - T_S$ ) were greatest for the indoor sheep at values of  $28.7^{\circ}\text{C}$  compared to  $25.9$  and  $26.4^{\circ}\text{C}$  for the control and outdoor sheep, respectively.

Mean heat production of the drug-free sheep during the exposure period was  $392 \text{ kcal/kg weight kg}^{0.75}/24 \text{ hr}$  for the indoor sheep,  $313 \text{ kcal/kg}^{0.75}/24 \text{ hr}$  for the control sheep and  $219 \text{ kcal/kg}^{0.75}/24 \text{ hr}$  for the outdoor sheep (Table 5). The indoor sheep had metabolic values as high as 433 units after two hours of exposure but this was not maintained and decreased to 418 units in the last thirty minutes of exposure. Fasting metabolic rate has been estimated at  $55 \text{ kcal/kg}^{0.73}/24 \text{ hr}$  for





TABLE 5 - Mean Metabolic Rate of Sheep Subjected to Severe Acute Cold Stress (-30°C)

Metabolic Rate kcal/kg 0.75/24hr		Times (hours)					
		0.5	1	1.5	2	2.5	3
Without Propranolol	Control	236	308	339	329	336	338
	Outdoor	182	202	227	238	238	234
	Indoor	294	374	416	427	433	418
MEANS							
With Propranolol	Control	214	284	312	310	326	323
	Outdoor	197	211	233	227	239	231
	Indoor	272	333	351	389	360	387

Resting Metabolic Rate (+18°C) kcal/kg<sup>0.75</sup> /24hr\*

Control 127  
Outdoor 137  
Indoor 137

Fasting Metabolic Rate 55kcal/kg 0.73 /24hr\*\*

\*Webster and Hicks, 1968b

\*\*Blaxter, 1962



adult sheep (Blaxter, 1962). The values from the present experiment are as high as eight times this estimated fasting rate. Alexander (1962) reported that the "summit metabolism" of new born lambs from 2 to 5 kg weight was five times the basal level of energy metabolism. Maximum energy expenditures in the present experiment have considerably exceeded this level of metabolism. Mean skin temperature of the outdoor sheep suggest that the intensity of cold stress was similar to that experienced by the control sheep. Metabolic expenditure for the outdoor sheep, however, was only 70% that of the control sheep. This lesser metabolic response was, in part due to the fact that the outdoor group had the greatest external insulation (Table 4). However, skin temperature measurements indicate that the outdoor group were subjected to a peripheral cold stress of comparable intensity to that experienced by the other groups. Consequently, some other compensatory mechanism must have been involved to maintain metabolism of the outdoor sheep at this lower level. Some indication of this is provided by the results obtained for tissue insulation in the three groups.

Tissue insulation values of 5.2 and 4.7 units for the control and inside sheep, respectively were determined. These values were consistent with those found during the preceeding winter for these sheep (Webster and Hicks, 1968b), and close to the mean value of 5.66 reported by Webster and Blaxter (1966). The outdoor sheep indicated tissue insulation values of 7.5 units. This was far above any normal values reported.



### Effect of Propranolol

The mean effects of propranolol on the body temperatures and heat production of sheep during acute cold stress are also shown in Table 4. The rectal temperatures of the control and outdoor sheep did not change significantly from the drug-free trials. The indoor sheep, however, demonstrated an increase of  $0.7^{\circ}\text{C}$  on rectal temperature above the former non-propranolol trials. The fall in mean rectal temperature after the maximal level had been reached was comparable for all groups ( $0.45^{\circ}\text{C}/\text{hr}$  for the control and outdoor sheep and  $0.6^{\circ}\text{C}/\text{hr}$  for the indoor sheep, Fig. 10). Comparison of these results with the drug-free experiments reveals that the effect of propranolol on rectal temperature change during cold exposure was greatest with the indoor group.

Difference in skin temperature between drug-free and propranolol treatments were inconsistent. Skin temperature in the outdoor and indoor groups was higher and in the control group it was lower when propranolol was administered (Table 4).

Energy expenditure for the control and the indoor sheep was significantly less ( $P < 0.01$ ) than in the drug-free trials. There was no significant difference in energy expenditure for the outdoor sheep after propranolol blockade.

Tissue insulation values with propranolol were greater than drug-free trials for the control and indoor sheep. Insulation of the tissues can change with the amount of superficial blood flow in superficial tissues and the amount of shivering. If either one or both of



these decreased, the insulation should be greater.

Heart rate during cold exposure was very much lower than in the drug-free sheep. However values were still maintained at a higher level than at rest. The control and indoor sheep demonstrated mean values of 118 and 112 beats / min respectively, while the outdoor sheep had a mean rate of 98 beats / min.

External insulation for all sheep was greater during propranolol infusion compared to the trials. In all cases, the drug-free trials were carried out before the propranolol treatments. Consequently the fleece had approximately a one week growing period. During the winter, fleece depth of the sheep increased approximately one mm. per week ( Webster and Hicks, 1968b). Although the increase in fleece was slight, the present data indicated increase in external insulation. External insulation is, however, a value which can change with physical qualities such as dampness and effect of wind. The effect of the increased insulation on metabolic expenditure in acute cold ( $-30^{\circ}\text{C}$ ) would have been very small.

### Discussion

Summit metabolism is the highest metabolic rate attainable at normal body temperature without voluntary muscular activity (Alexander, 1962). The ability to maintain normal body temperatures under stressful conditions can depend on the animal's environmental past history (Hsieh et al., 1966). All of the sheep in this attained exceedingly high levels of metabolic rate in an air temperature of  $-30^{\circ}\text{C}$ .

Alexander stated that summit metabolism for newly born lambs was five times that of the "basal" levels. The present results show that the indoor sheep achieved a metabolic level at least eight times





the estimated fasting level. Webster and Hicks (1968b) described an increased resting metabolic rate in these sheep during adaptation to their environment. Consequently, basal level of metabolism should have also been greater than the estimated fasting level. Although these increases in metabolic rate for the indoor sheep are high, incremental increases would not then be as great as suggested. Metabolic responses of the control sheep to the cold were five times greater than the estimated fasting level. These sheep had attained relative metabolic levels similar to that reported by Alexander (1962).

The outdoor sheep attained metabolic rates which were four times greater than the estimated fasting levels. However, these sheep also had shown adaptations during the previous winter resulting in higher resting metabolic rates (Webster and Hicks, 1966b). Incremental increases in heat production would, as with the indoor sheep, appear to be less than that predicted from estimated fasting values.

Results of skin temperature indicate that all of these sheep were affected similarly by the cold stress. However, the mean skin temperature for the indoor sheep was  $2.5^{\circ}\text{C}$  less than the other sheep and the temperature differential ( $T_R - T_S$ ) was greater for these indoor animals (Table 4). Thus it appears that these indoor sheep combated the cold stress by increasing heat production rather than decreasing avenues of heat loss. Although rectal temperature had increased and then decreased in the indoor sheep, the absolute level after three hours of cold exposure was only  $0.2^{\circ}\text{C}$  lower than the pre-exposure temperature. Summit metabolism had not then been achieved by the indoor sheep.

The rate of decrease of rectal temperature for the outdoor



sheep was similar to that of the control sheep. This would tend to indicate, according to Alexander's definition, that summit metabolism had been reached in these animals. However, heat production for these outdoor sheep was at a level only 70% that of the control sheep.

It appears most unlikely that these sheep were, in fact, producing heat to their maximum capacity. Evidence of their metabolic acclimatization to previous winter exposure (Webster and Hicks, 1968b) and subjective impressions formed on the behavior of the sheep in the cold room, both suggested that these animals were under less stress at  $-30^{\circ}\text{C}$  than the sheep in the other groups. One might postulate that acclimatization of the outdoor sheep during the previous winter had conditioned them to display a smaller metabolic response to a given intensity of peripheral and central cold stress and thus tolerate some degree of heterothermia. The advantages of this in regard to energy conservation in a fluctuating cold environment are obvious.

The effect of shearing appeared to increase the tissue insulation of the outdoor sheep. It was reported (Wodzicka, 1958) that shearing affected the thickness of the skin considerably. Fourteen days after shearing, skin thickness had increased an average of 28.5% compared to thickness before shearing. The outdoor sheep used in the present study had been sheared about ten days before the exposure trials were carried out. Nonetheless, the control animals had been sheared about fourteen days before being subjected to the extreme cold without great change in tissue insulation. Some other explanation of the high tissue insulation in the outdoor sheep must be sought. Increased tissue insulation could be brought about by an exchange of muscular shivering, which normally is the first line of



defensive heat production in warm-acclimated animals, to increased non-shivering tissue energy metabolism. This non-shivering component could maintain the body core temperature and be more efficient for heat conservation. Jansky (1966) indicates that a large percentage of the non-shivering thermogenic effects of rats is from non-contractile energy exchanges in skeletal muscle. During the present trials, observed shivering and general discomfort appeared to be less for these outdoor animals compared to the other animals. A similar method of non-shivering thermogenesis may be evident with these sheep adapted to many fluctuating environmental changes.

Propranolol (1.0mg/kg) treatment did not affect tissue insulation of the outdoor sheep. The control and indoor animals both had an increase in tissue insulation. Propranolol inhibits beta-adrenergic control of vasodilation in blood vessels to the peripheral regions and to the skeletal muscle. Consequently, any peripheral blood flow as a source of heat loss to the sheep was restricted. However, no evidence at present, suggests that the control of heat loss via peripheral vasodilation in the sheep is great.

Propranolol treatment decreased the metabolic response of the control sheep by about 6% and the indoor sheep by about 12%. Metabolic response with propranolol for the outdoor sheep did not change although sympathetic control of energy metabolism was shown to be very limited. As in the previous experiment, some control exists. Increased metabolic response for the control and indoor sheep above those values for the propranolol treatments was due to sympathetic intervention. Since the indoor animals were acclimated, and although summit metabolic levels had not yet been achieved, a greater sympathetic involvement is evident.





This is qualitatively similar to the responses in white rats, which have been acclimated to cold temperatures and involves increased non-shivering thermogenesis produced by an increase in sympathetic control of energy metabolism (Jansky, 1966). The acclimated rat has a greater sensitivity to noradrenaline induced thermogenesis than the warm-adapted rat. Increased metabolic response with cold-acclimation in these sheep incorporates some greater sympathetic involvement than in the warm acclimated sheep in cold stress.

Propranolol treatment effectively inhibited sympathetic control of heart rate for a minimum time of two hours after infusion. Heart rate equilibration was usually achieved by one to one and one half hours of exposure at rates which were higher than those of the initial exposure by about 10% (Appendix 9). Inhibition of sympathetic control of cardio-acceleration suggests that the withdrawal of vagal inhibition is high for sheep under acute cold stress. Decreasing vagal inhibition has been shown to increase the heart rate performing light exercise in man (Robinson et al., 1966). Nothing has been reported to the present time on the effects of vagal inhibition of heart rate in sheep during cold exposure. If decreased vagal inhibition is responsible for increasing heart rate in the cold without sympathetic intervention, then the intrinsic heart rate of sheep must be at a level of 110 beats/min or greater.

#### Summary

1. Propranolol (1.0 mg/kg) inhibits the maximal heat production of warm-and cold-acclimated sheep in severe cold.

2. Propranolol (1.0 mg/kg) inhibits the total increase in heart rate of sheep in severe cold exposure (-30°C). Heart rates of about 100 beats/min. were shown.





3. Warm-acclimated sheep exposed to severe cold try to maintain normal body temperatures by increasing heat production.

4. Winter-acclimatized sheep exposed to severe cold react by decreasing body temperature and increasing metabolic rate at a level less than that of warm-acclimated sheep in a similar environment.

5. Cold-acclimated sheep exposed to severe cold maintain body temperature. They were capable of increasing heat production to levels greater than that of warm-acclimated sheep in a similar environment.



## V. Experiment 3

### Effects of Direct Nerve Stimulation and of Feeding on Heart Rate in Sheep with Pharmacological Cardiac Denervation

#### Objective

The former experiments describe the effect of propranolol, a beta-adrenergic blocking agent, on the cardioacceleration produced with isoproterenol, eating and cold exposure. These stimuli used have been relatively non-specific for the control of heart rate because of the possible physiological interaction from other sources; therefore, it was felt that a specific electrical stimulation, at a physiological level, of the efferent nerves to the pharmacologically blocked heart would better serve to demonstrate specific adrenergic and also cholinergic blockade.

It has been shown that beta-adrenergic control was not primarily responsible for increasing heart rate during eating in sheep. Preliminary trials of complete heart blockade in the non-anaesthetized sheep were carried out to estimate interaction of, basically, a denervated heart during rest and eating.

#### Methods

##### A. Anaesthetized Sheep

Three adult Suffolk sheep and two Columbia lambs were used for these trials. The animal were anaesthetized with nembutal and prepared for surgical isolation of the left and right vagi in the neck region and isolation of the left and/or right stellate ganglion(s). Preparation of the animals included catheterization of a carotid artery for



blood pressure measurements and cannulation of the trachea for possible respiratory control and regulation. Measurements of carotid arterial blood pressure were monitored with a carrier pre-amplifier<sup>1</sup> (350-1100 Hz) by use of a pressure transducer<sup>1</sup>. The catheter, filled with heparinized saline, was connected to the pressure transducer with a coupling and a three-way tap. This made it possible to clear any obstruction from the catheter with saline. The electrocardiogram (ECG) was monitored on the Sanborn<sup>1</sup> (preamplifier 350 - 2700 Q) using surface or skin electrodes (Section II) while heart rates could be determined either from integration of the ECG peaks (preamplifier 350 - 3400 A) or by counting the individual systole-diastole fluctuations of blood pressure. The second method was generally used when nerve stimulation was under weigh due to the interference from the electrical stimulator. However, it was found that at very low voltages heart rate could still be determined from the ECG.

#### Vagal Stimulation

After the vagi had been isolated from the surrounding tissue the left vagus was severed and stimulated on the peripheral end with an electrical impulse from an inductorium great enough for temporary cardiac arrest. This stimulation was maintained for fifteen seconds. The central end of this vagal trunk was similarly stimulated. A similar technique was carried out with the right vagus (this being the major vagal trunk of cardiac inhibition to the sinus-atrial node). Atropine (0.05 mg/kg, i.v.) was injected through the jugular catheter over a one-minute period. A twenty minute interval was allowed before

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<sup>1</sup>Hewlett-Packard (Canada) Ltd., Montreal, Quebec.



electrical stimulation

#### Surgical Isolation of the Left and Right Stellate Ganglia

It has been shown (Waites, 1957) that the main controlling sympathetic mechanism of heart rate in the sheep is via the right chain of the sympathetic nervous system. The main ganglion is the right stellate ganglion. Isolation and stimulation of both stellate ganglia were carried out in some experiments followed by propranolol treatments and electrical stimulation. However, due to the length of time involved for isolation of both vagi and stellate ganglia when the animal was under anaesthesia (which could go as long as twelve hours), some trials were carried out with specific right stellate isolation and stimulation only.

Isolation of the right stellate ganglion: This was accomplished by first drawing the right foreleg backwards and locating the first rib just forward of the scapula. A lateral incision, of about three inches, was made through the skin in the immediate area and the muscle in the region was separated to reveal the right vertebral artery and vein just before they disappeared under the rib (Fig. 11). The periosteum was then loosened and removed with a Stille osteotome and a Doyen rib raspator<sup>2</sup> all of the way around the rib from the head for about one inch along the lateral region of the body. Much care was required to avoid any pneumothorax which, if produced, tended to make isolation of the ganglion difficult. The section of the rib which had been cleaned was then cut with a Gluck rib shear<sup>2</sup> and removed. The

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<sup>2</sup>The Stevens Co. Ltd., Calgary.







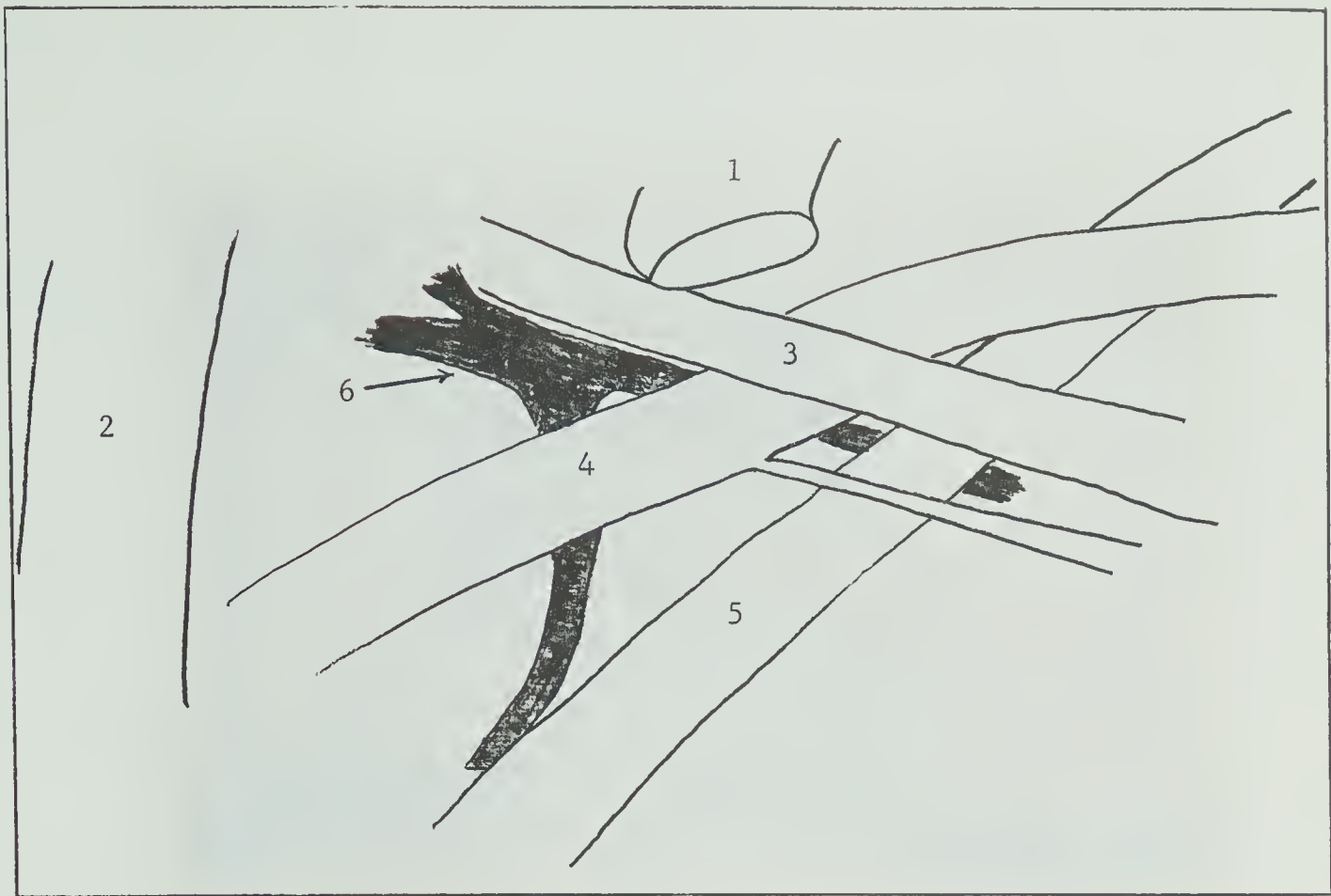


FIGURE 11 - The right stellate ganglion of the sheep with the first rib removed.

1. Rib 1. cut out
2. Rib 2.
3. Phrenic nerve
4. Vertebral artery
5. Vertebral vein
6. Stellate ganglion





FIGURE 12 - Dissection in left thoracic region of sympathetic chain and heart.

1. Left stellate ganglion on paper background
2. Phrenic nerve
3. Vagus nerve
4. Subclavian artery
5. Left atrium of the heart



periosteum and the outer layer of pleura were left intact. The periosteum was then removed with a pair of forceps and interfering fat and lymph was removed for easier access to the region. In this region the sympathetic chain appears near the vertebral artery and vein beside the right phrenic trunk. The ganglion is located because of its triangular shape just posterior to the location of the first rib. The central and peripheral parts of the sympathetic chain were then severed leaving the branch to the sinus-atrial node intact. The ganglion was tied with a piece of suture making it available for stimulation.

Isolation of the left stellate ganglion: This was carried out in much the same way. The points of reference are quite similar to those on the right side. The ganglion is dorsal to the branching of the branchial and arterial arteries. When the rib was removed the stellate ganglion appeared posterior compared to the ganglion of the right side. This places the ganglion almost between the first and second rib.

#### Electrical Stimulation of the Stellate Ganglia

After the respective stellate ganglia had been isolated an electrical stimulation (inductorium of Grass Electrical Stimulator Model SD5<sup>3</sup>) was administered for fifteen seconds to produce a physiological increase in heart rate to not more than 250 beats/min. An impulse of 4 volts, 20 pulses/sec, 5 msec duration was possible made with the Grass stimulator. After the effect of stimulation had been

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<sup>3</sup> Grass Inst. Co., Quincy, Mass., U.S.A.



determined, propranolol (0.5 mg/kg) was infused over a one minute time period. Stimulation of the respective ganglia was then repeated.

The two trials with the lambs included inhibition-time studies on the effect of propranolol blockade. The right stellate ganglion was electrically stimulated every fifteen minutes for a total period of four hours after propranolol infusion. After each period of heart rate adjustment, (i.e., heart rate at the prestimulation level), which usually required one minute, isoproterenol (0.2  $\mu$ g/kg, i.v.) was injected. A comparison of the two types of stimulation on increased heart was made.

#### B. Conscious Sheep

##### Cardiac Pharmacological Blockade in Conscious Sheep

Studies were carried out to investigate the effect of propranolol (0.5 mg/kg) and atropine (0.05 mg/kg) on the heart rates of resting and eating sheep. Two Columbia-Lincoln lambs each weighing 20 kg were infused (i.v.) with a saline preparation of the drug over a fifteen minute period. Following such a treatment in dogs (Jose and Stitt, 1967) and in man (Jose, 1966) the heart resembles a classical isolated heart-lung preparation. Heart rates were recorded every five minutes for an interval of four hours. An estimate of resting heart rate was obtained during the thirty minutes before infusion.

The same five Lincoln male sheep used previously for the eating and cold experiments were used for the pharmacological blockade studies during eating. Two of the animals, Happy(T) and Dopey(T) had been thyro-parathyroidectomized at least sixty days before these trials. It





is usually impossible to remove the thyroid glands of a sheep without, at the same time, removing the parathyroid glands. These thyro-parathyroidectomized animals were maintained on the same ration as the other animals (1000 gm/day) but were supplemented with calcium in their drinking water (1.5 grams of calcium lactate/wt.(kg sheep/day) to avoid tetany, osteomalacia or any other calcium deficiency which could occur with loss of the parathyroid gland. Heart rate measurements were recorded for a prefeeding period of one hour and during this time the drug was administered. Propranolol and atropine were prepared as before and infused over a fifteen minute period. After an additional fifteen minutes alfalfa-brome hay was given to the sheep and heart rates were recorded for a maximum eating time of one hour. The feed was then removed at this time and measurements were continued for another hour. Duplicate trials were conducted for each animal.

## Results

### A. Anaesthetized Animals

Levels of electrical stimulation were determined during some initial trials which would just arrest pulsation of the heart when applied to the peripheral end of the right severed vagus and not to increased above 250 beats/min when applied to the isolated right stellate ganglion.

#### Electrical Stimulation of the Vagi(X) Nerves

Electrical stimulation of the peripheral end of the right severed vagus nerve for a fifteen second interval resulted in cardiac arrest (Table 6 ). Blood pressure decreased substantially as well



TABLE 6 . Stimulation of the Vagi (X) Nerves Before and After Atropine Injection

	Heart rate (beats/min)		Blood pressure (mm/Hg)	
	pre-inhibition	atropine	pre-inhibition	atropine
Anaesthetized	177+15*	177+15	120/85 (+5)*	130/95 (+4)
$\Delta$ Heart rate (beats/min)				
Central right	-12+2**	+6+2	125/95(+10)	135/95(+10)
Peripheral right	complete block	+2+1	↓ ↓	
Central left	-8+3	+6+1	120/90 (+5)	145/110(+15)
Peripheral left	decreased heart rate or complete blockade	+6+0	↓	115/80 (+5)

\*S.D.

\*\*S.D. of differences.

Measurements of heart rate (beats/min) and blood pressure (mm/Hg) after electrical stimulation of the central and peripheral ends of severed vagi before and after atropine (0.05 mg/kg, i.v.) injection.



from anaesthetized values of 120/80<sup>4</sup> to unrecordably low levels. Central right vagus electrical stimulation decreased heart rate by 12 beats/min and blood pressure increased slightly to 125/95. Because of the occurrence of increased vomiting and salivation, stimulation of the central ends of the vagi were kept to a minimum in order to avoid increased complications. Similar stimulations of the left vagus resulted in a decrease of 8 beats/min for the central end and again cardiac blockade with peripheral stimulation. Blood pressure was similar to before. Usually after the arresting stimuli, heart rate and blood pressure recovery to pre-stimulation levels would require about two minutes.

A twenty minute period followed atropine injection (0.05 mg/kg , i.v.) which allowed the total effect of blockade to develop. Right peripheral vagal electrical stimulation was completely inhibited by heart rate and blood pressure changed only to 120/85. Central electrical stimulation of this same nerve trunk resulted in an increase in heart rate of 6 beats/min above the anaesthetized level and a blood pressure of 135/100. Peripheral stimulation of the left vagal trunk increased heart rate 6 beats/min but blood pressure tended to decrease to 115/80. Stimulation of the central trunk of the left vagus increased heart rate 16 beats/min and blood pressure to 145/110.

#### Electrical Stimulation of the Stellate Ganglion

After right stellate ganglion isolation anaesthetized heart rate was 120 beats/min. A change in heart rate of 103 beats/min

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4. Systolic-diastolic blood pressure in mm. Hg.



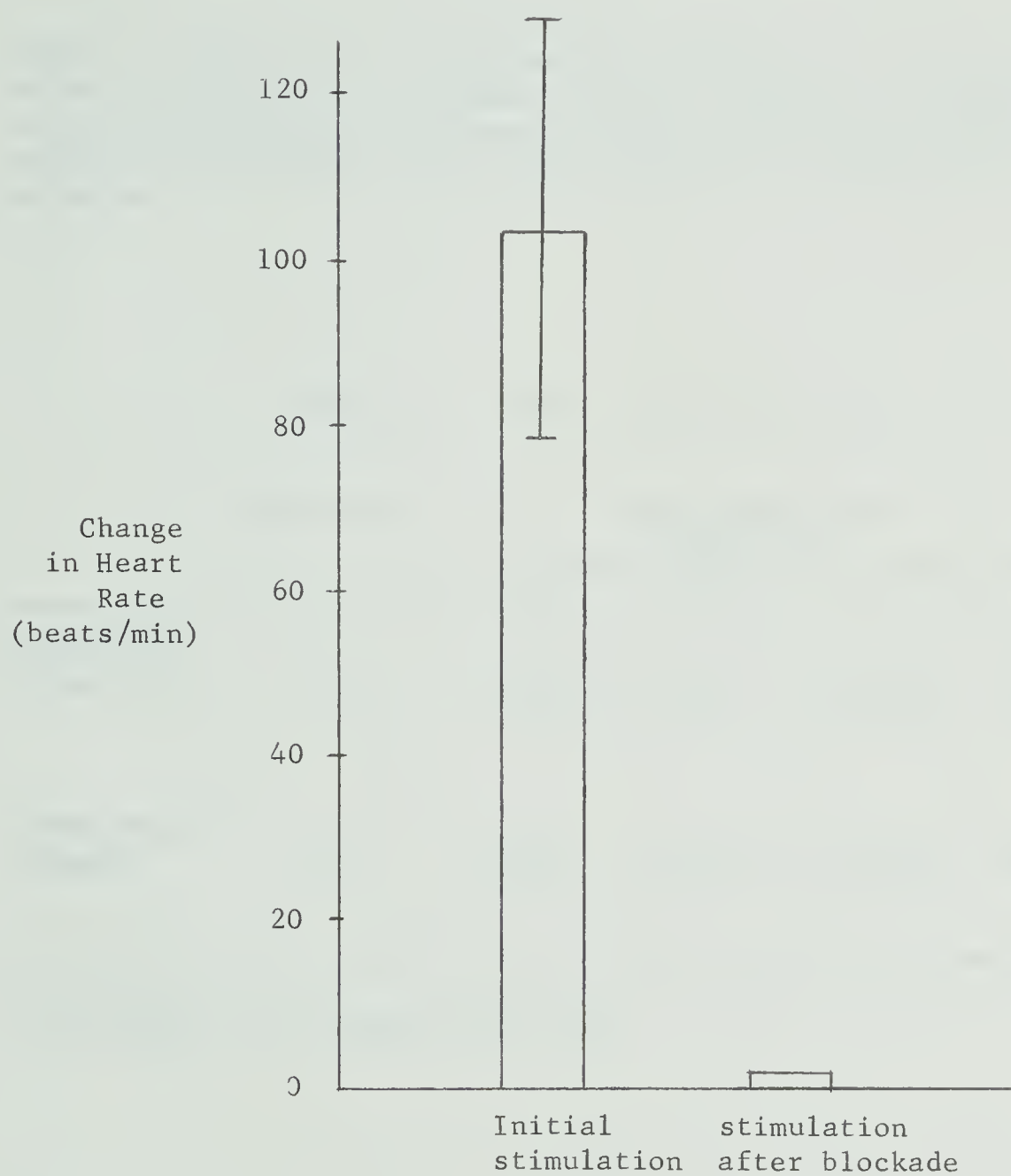


FIGURE 13 - Direct electrical stimulation of the right stellate ganglion for three sheep. Bar value is standard deviation.





TABLE 7 - Time effect of propranolol inhibition on the heart with electrical stimulation on the right stellate ganglion and isoproterenol injection ( $0.2 \mu\text{g/kg}$ ). Values are increases in heart rate above the resting anaesthetized levels before and after propranolol ( $0.5 \text{ mg/kg}$ ) infusion.

Change in Heart rate (beats/min)					
	Propranolol	Post-propranolol			
		Time (hr)			
		1	2	3	4
Electrical stimulation	120	+2(98)*	+6(95)	+40(66)	+65(46)
Isoproterenol $0.5 \mu\text{g/kg}$	110	+5(95)	+16(87)	+41(65)	+84(23)

\*% blockade of increased heart rate.



resulted from electrical stimulation. After propranolol (0.5 mg/kg) infusion heart rate increased to 140 beats/min and tended to be quite consistent at this level. Electrical stimulation comparable to that given before propranolol was effectively inhibited with repeated stimulations (Fig. 13)

After stellate isolation in sheep 97X, with intact vagi, anaesthetized heart rate was 120 beats/min. A change in heart rate of 120 beats/min resulted from electrical stimulation and an increase of 110 beats/min with isoproterenol (0.2  $\mu$ g/kg, i.v.) injection. After electrical stimulation and isoproterenol injection the anaesthetized heart rate was 140 beats/min. Electrical stimulation and isoproterenol injection values were pooled for each hour. After one hour, electrical stimulation increased heart rate only +2 beats/min which indicated 98% blockade to the heart. Isoproterenol injection increased the heart rate +5 beats/min or 95% blockade (Table 7 ). Thus a high level of beta-adrenergic inhibition was still apparent.

A real difference in blockade break through came two hours after propranolol infusion. For the three hour period electrical stimulation demonstrated 66% blockade and isoproterenol 65% blockade.

#### B. Conscious Animals

All of the normal intact sheep and lambs infused with the propranolol (0.5 mg/kg)-atropine (0.05 mg/kg) responded with an immediate increase in heart rate from a mean of 60 beats/min to 110 beats/min for the Lincoln males (Fig.14a) and a mean of 60 beats/min to 90 beats/min for the two lambs (Fig.14c). This level of heart



FIGURE 14a

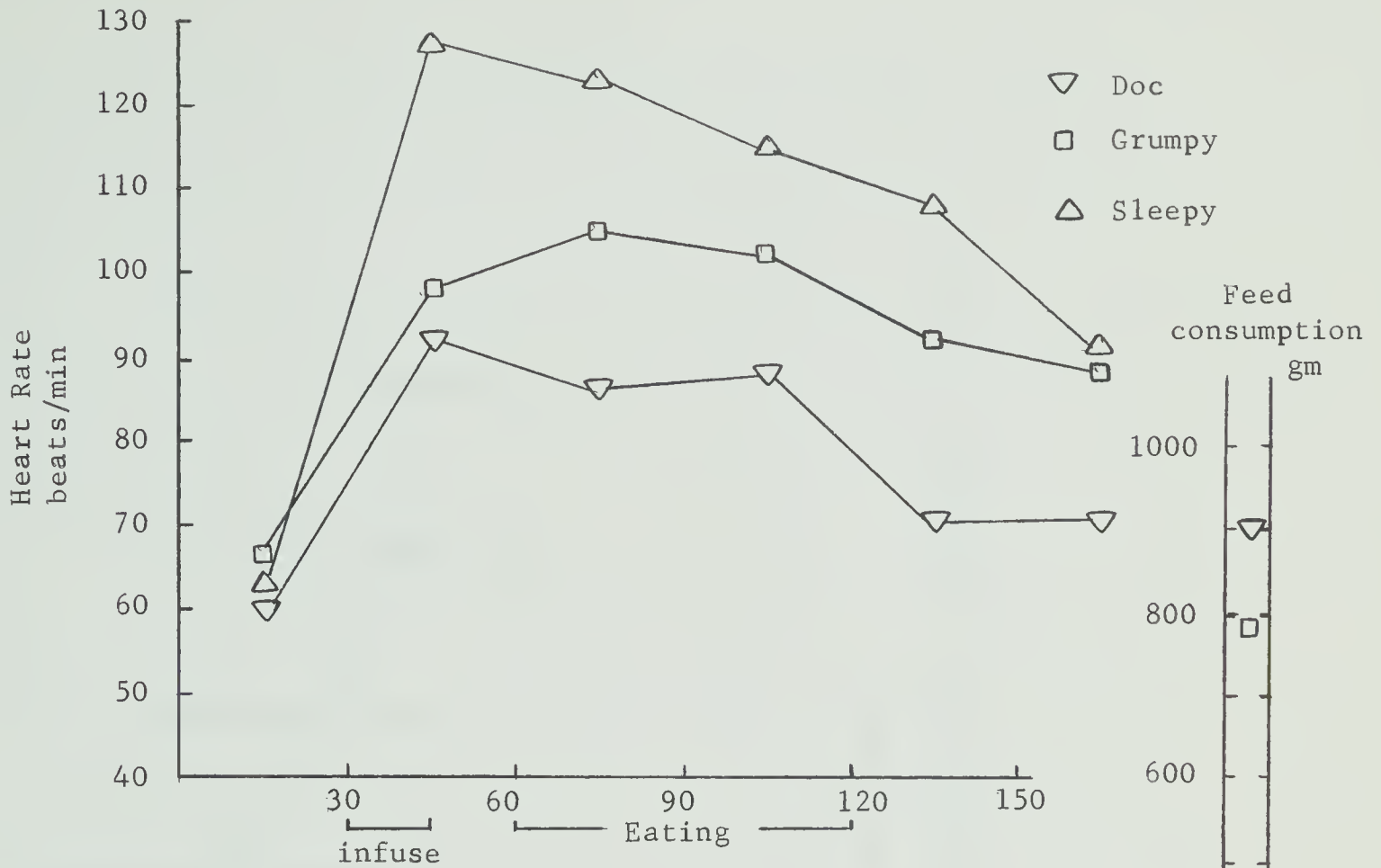


FIGURE 14b

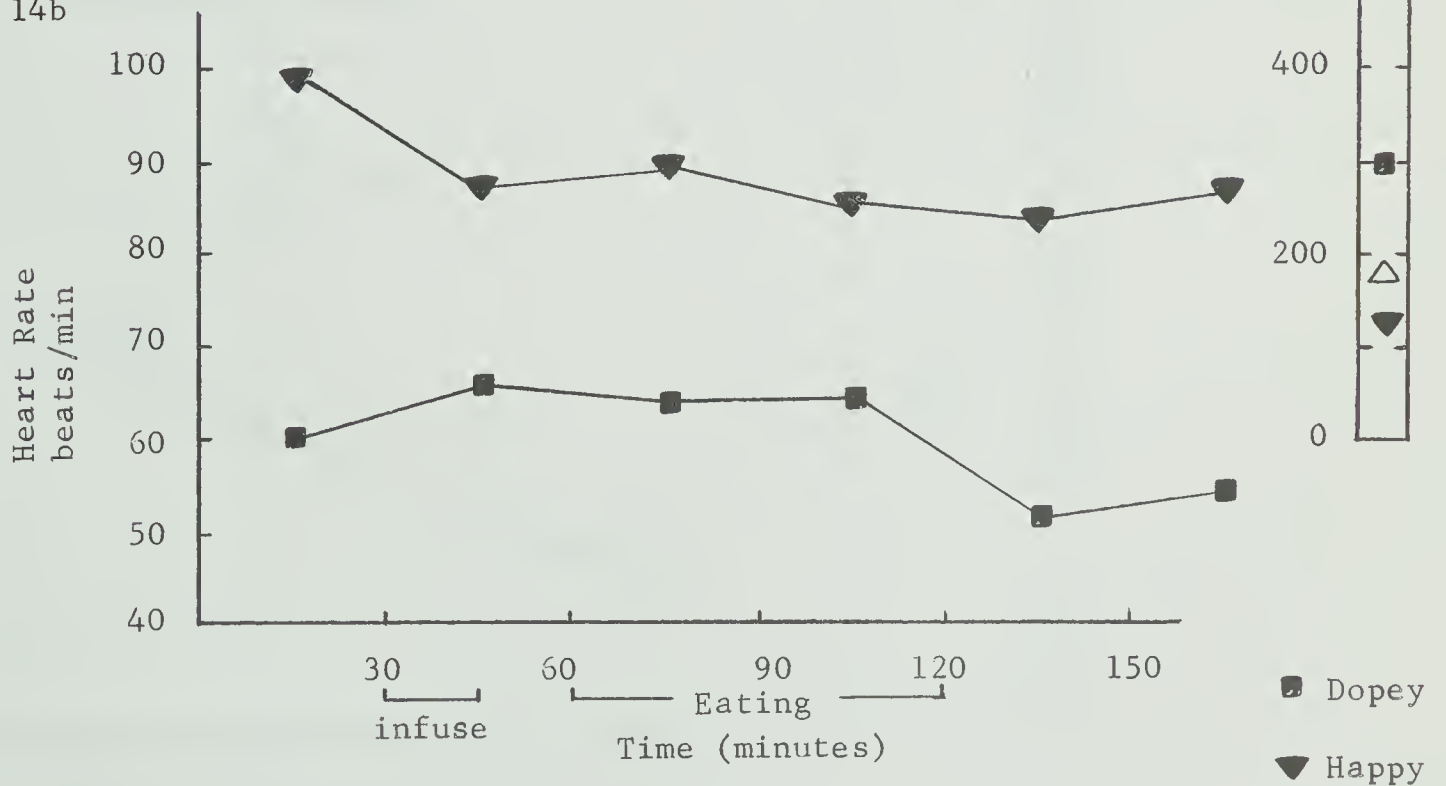


FIGURE 14 - Heart rate in euthyroid (Figure 14a) and thyroidectomized sheep (Figure 14b) during eating with pharmacological blockade.



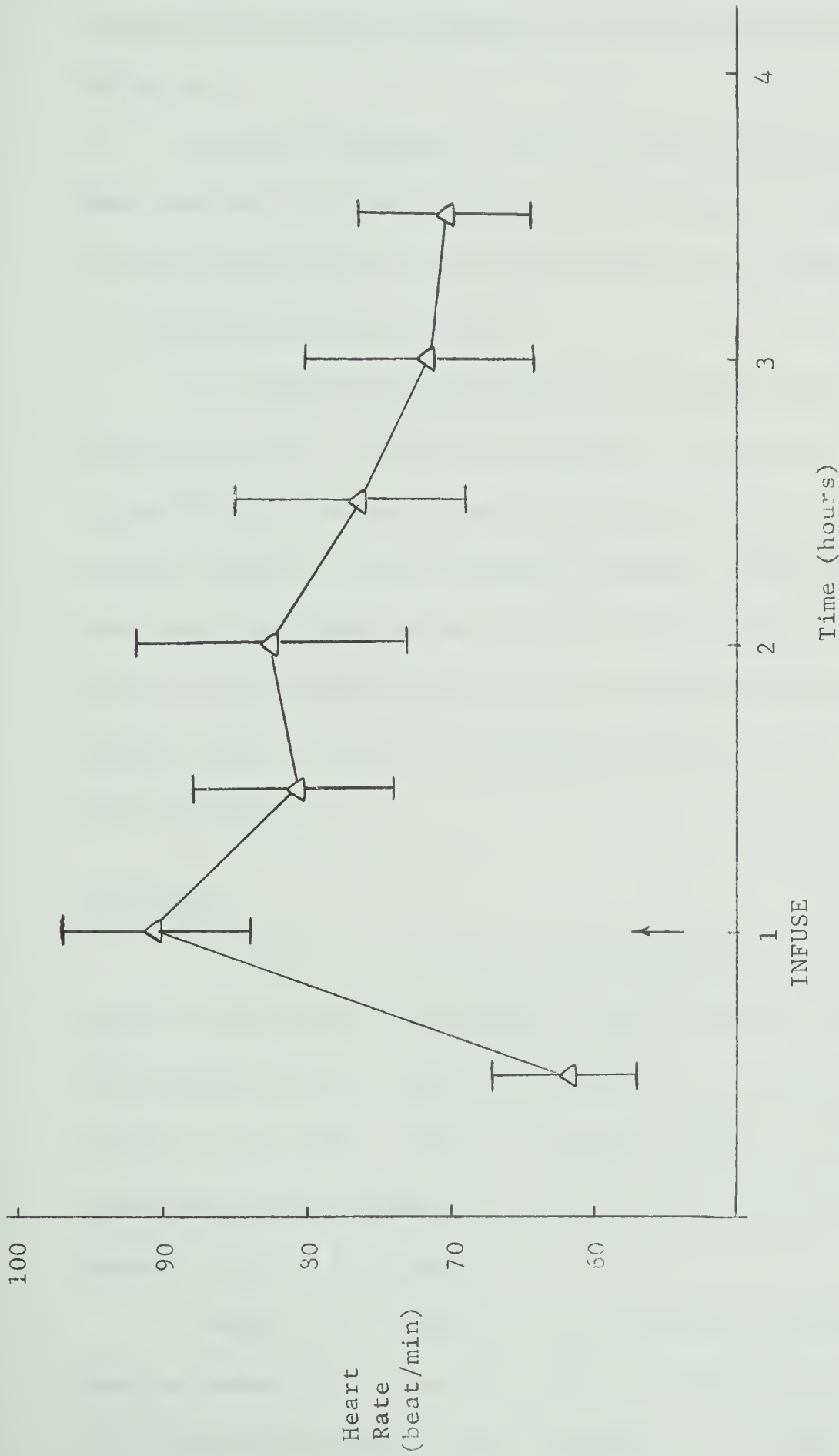


FIGURE 14c - Pharmacological isolation of the heart in 2 lambs (3 trials for each). Bars are standard deviation for all trials.





rate in the lambs decreased to 81 beats/min during the first thirty minutes and steadily decreased to 70 beats/min two hours following infusion.

During the feeding trials the heart rates of normal, intact sheep did not increase above the post-infusion levels. These heart rates were generally maintained during eating and began to decrease to levels above those at rest.

The thyro-parathyroidectomized animals had a different response to the drug. Dopey(T) indicated a rise in the mean heart rate in the thirty minutes following infusion from 60 to 65 beats/min whereas Happy(T), during the same interval demonstrated a fall in mean heart rate from 98 to 86 beats/min. During eating no significant change in heart rate was evident for Happy(T) but Dopey(T) did decrease heart rate by 10 beats/min during the first thirty minutes after the meal.

### Discussion

The level of propranolol (0.5 mg/kg) used in the trials on anaesthetized animals effectively inhibited the increase in heart rate following direct electrical stimulation of the cardioaccelerator nerves to the heart. This was the same level of propranolol which inhibited increased heart rate during the cold exposure and isoproterenol trials of Experiment 1.

Results from Sheep 97X illustrated the similarity between the dose of isoproterenol and the level of electrical stimulation selected for cardioacceleration. The decrease in the blocking effect of



propranolol with time in this animal was also very consistent for both types of stimulation. Inhibition of increased heart rate was maintained in the range of  $90 \pm 5\%$  for two hours as indicated by the respective stimulation (Table 7). During the third hour, the effectiveness of blockade decreased to 65%. This was similar to these results of isoproterenol injection in Experiment 1.

The tetanizing electrical impulse used for vagal stimulation was of an intensity great enough to cause cardiac arrest. This heart rate inhibition was demonstrated by stimulation of peripheral ends of either of the two vagal trunks. Individual results did, however, tend to confirm that the main parasympathetic control of the heart is via the right vagal neural trunk.

The concentration of atropine (0.05 mg/kg) used to inhibit vagal control of the heart rate in conscious sheep was comparable to those levels used for vagal inhibition in man (Cumming and Carr, 1967). The effect of direct electrical stimulation of the peripheral vagi in anaesthetized sheep was blocked after atropine had been injected. Consequently, pharmacological blockade with propranolol (0.5 mg/kg) and atropine (0.05 mg/kg) was very effective during direct electrical stimulation of the respective nerves.

The results of these experiments do not permit an exact evaluation of the relative importance of direct inhibition by atropine of tonic vagal impulses to the heart and of the possible direct chronotropic effect of propranolol in the sheep. It is possible that propranolol could inhibit, slightly, parasympathetic and well as sympathetic control of the heart. Interactions between the effects



of propranolol and atropine on the heart remain to be resolved and are presently under study. Nonetheless, it can be concluded that treatment with atropine and propranolol did produce effective pharmacological blockade of autonomic control of the heart for two hours after injection.

The amount of eating activity was not uniform for all animals. Grumpy ate a maximum of 1000 gm of hay while minimum values of 150 gm for Happy(T) and Doc were recorded (Figure 14). Thus it could be expected that the activity of eating would be far greater for Grumpy than some of the other sheep due merely to the volume consumed. Once the intact sheep began eating, isolated heart rates did not increase but did manage to maintain a plateau of 102 beats/min. Over a similar period of time the heart rates of lambs, which were not offered food, did not change. Figure 15 compares the changes in heart rate from resting levels for intact sheep, thyro-parathyroid-ectomized sheep and lambs.

The period for eating, refers to adult sheep only. Relative changes for the fed intact sheep and lambs not offered food were similar. Further work on the effects of pharmacological blockade of the heart in sheep is presently under study.

Resting heart rates for dogs which had surgical cardiac denervation were about 100 beats/min and increased to peak values of 150 - 170 beats/min with exercise (Donald and Shephard, 1963). This increase however, could have been due to increased amounts of adrenaline or of catecholamines from nerves other than those directly serving the heart.



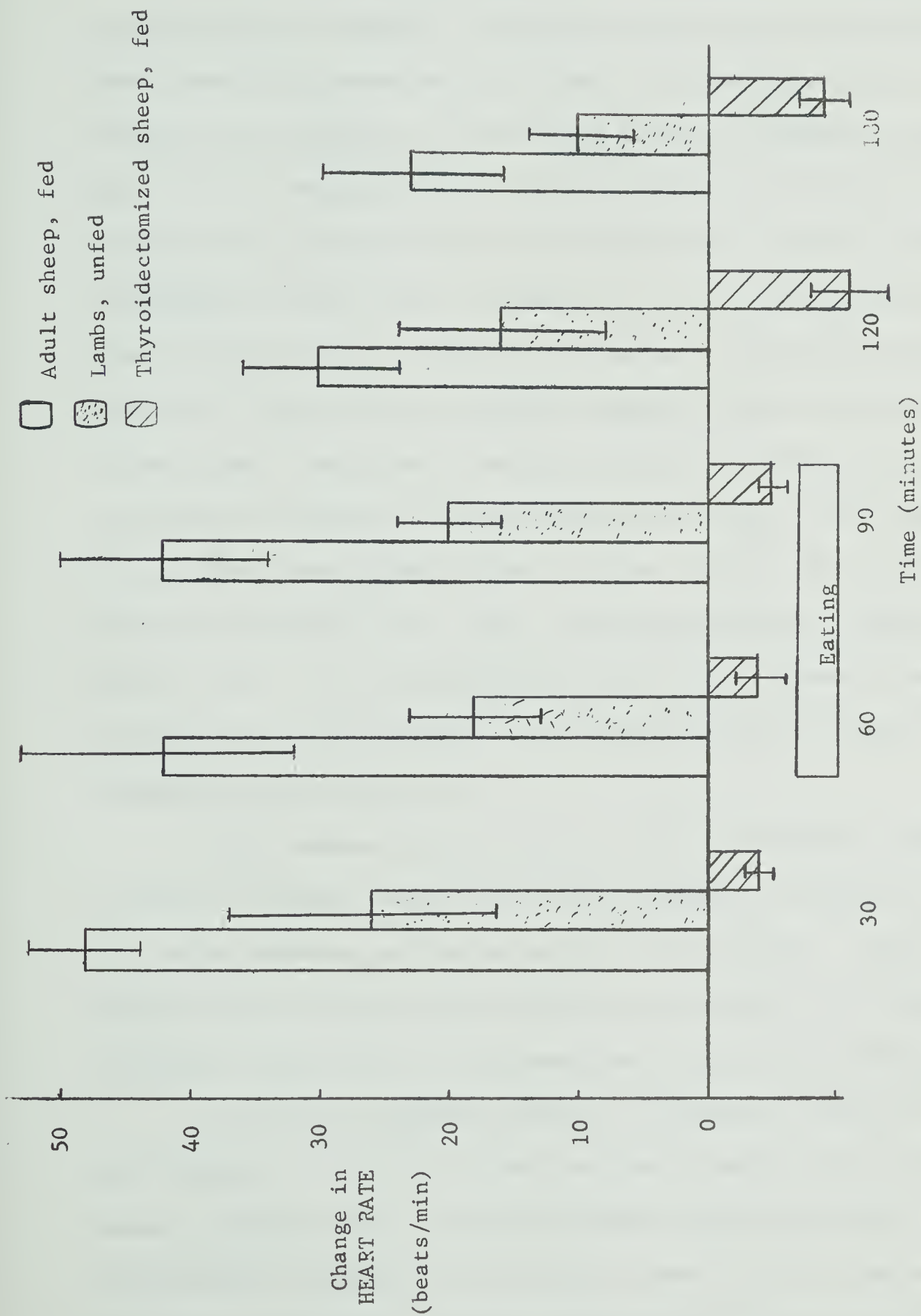


FIGURE 15 - Change in heart rate from rest for propranolol-atropine treated euthyroid sheep and lambs and thyroidectomized sheep respectively. Only the adult sheep were offered feed. Variations are standard deviation of change in heart rate.







A previous experiment had been carried out with Grumpy using atropine only (0.1 mg/kg). Increases in heart rate were recorded from a mean of 65 beats/min to a high of 124 beats/min during the infusion to an average of 87 beats/min after the infusion had stopped. The effect of eating increased the heart rate to 154 beats/min for the first thirty minutes after eating had begun. The heart rate was maintained at a high level throughout the eating process but did tend to drop off in the last thirty minutes to an average of 140 beats/min. After the food had been removed, heart rate decreased to 98 beats/min. The effect of atropine block was confirmed at this time with an injection of acetylcholine (0.4 mg/kg). This sheep was an exceptional animal in that atropine infusion did not seem to decrease his urge to eat. Other animals demonstrated a decreased desire to eat. This was undoubtedly due to the blockade of salivary secretion and parasympathetic rumen-reticular motility which normally accompany eating.(Figure 16).

Total pharmacological isolation, then, stimulates the effect of eating in normal sheep in that the incremental increase in heart rate after blockade and during eating is very similar. One might conclude that decreased vagalinhibition is largely but not necessarily completely responsible for increased heart rate during eating.

It has been suggested (McDivitt, 1968) that thyroid hormone acts directly on the heart of man and that a fall in the circulating amount produced either by thyroid hormone suppressing drugs or by thyroidectomy reduced the resting pulse rate and the incidence of paroxysmal tachycardia. Thyroid hormone did not potentiate the action



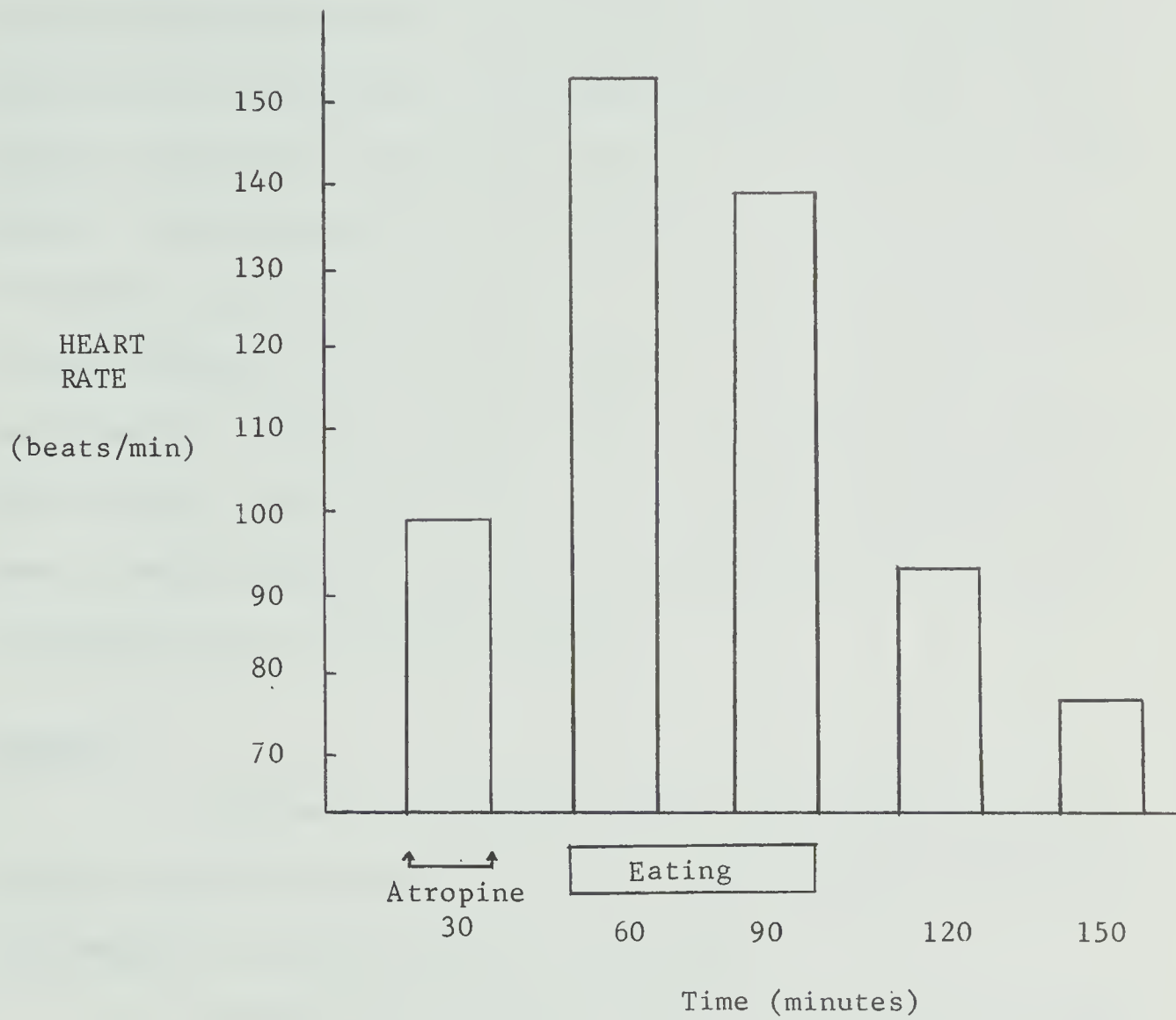


FIGURE 16 - Heart rate for Grumpy before, during and after eating with atropine infusion.



of catecholamines in the heart of man or dog (Margolius, 1965). No effect of thyroid hormone on the vascular activity of catecholamines in the dog has also been suggested (Johnson, 1968). In the present experiment, thyroidectomy did not alter resting heart rate. However, following pharmacological blockade, heart rate did not increase at rest or during eating. This contrasts sharply with the results offered in the euthyroid animals. It is reasonable to conclude that in sheep, thyroid hormone has a stimulating effect on the intrinsic rate of the heart. In the normal resting state, however, a marked degree of vagal tone maintains heart rate about 40 beats/min below its intrinsic rhythm.

#### Summary

1. Propranolol (0.5 mg/kg) inhibited the effect of physiological electrical stimulation to the cardio-acceleratory nerves in the sheep for a minimal period of two hours.

2. Atropine (0.05 mg/kg) effectively inhibited the effect of direct electrical stimulation to the efferent vagi to the heart.

3. Pharmacological blockade with propranolol (0.5 mg/kg) and atropine (0.05 mg/kg) showed a resting heart rate of about 100 beats/min for sheep.

4. Euthyroid sheep with pharmacological blockade did not increase heart rate with eating above the isolated intrinsic level.

5. No difference in resting heart rate was shown between euthyroid and thyroidectomized sheep.



6. Thyroidectomized sheep with pharmacological blockade did not increase resting heart rates above the isolated intrinsic level. No effect of eating on heart rate was observed in these animals.





## GENERAL DISCUSSION

The individual results of the three main experiments have already been discussed in detail. However, several topics emerge from the entire project whose implications and applications permit general discussion.

### Heart Rate and Energy Expenditure in Normal Sheep

Increased metabolic energy demands of eating and cold stress have been shown to be highly correlated with increased heart rate by Webster, (1967) who proposed that heart rate telemetry could be used as a technique for estimation of the energy expenditure of the individual grazing animal.

Table 8a shows the linear regressions of heart rate and metabolic rate for five sheep with fleeces when exposed to air temperatures between  $-20^{\circ}$  C and  $+10^{\circ}$  C. A large amount of variation was shown between sheep. Mean regression values were calculated for all sheep from average heart rates and the average heat productions for each time period.

Table 8b shows the linear regression values of the same individual sheep during eating. Two feeding trials were carried out (Experiment one). Variation in linear regression between the two feeding trials was evident for the same animals. Mean regression values were determined for all sheep for each trial during eating from average heart rates (Y) and average heat production (X) for each time period. The two mean regression formulae,  $Y=0.68X - 1.6$  and  $Y=0.69X - 4.0$ , are similar for both feeding



TABLE 8 a - Regression of Heart Rate and Heat Production During Acute Cold.

Sheep	Regression*	S.E. of Regression coefficient
DC	$Y = 0.51 X + 22.8$	$\pm 0.101$
DY	$Y = 0.56 X - 5.8$	$\pm 0.047$
SY	$Y = 1.23 X - 9.07$	$\pm 0.076$
GY	$Y = 0.53 X + 1.20$	$\pm 0.106$
HY	$Y = 0.98 X - 7.04$	$\pm 0.75$
MEAN	$Y = 0.85 X - 25$	$\pm 0.055$

TABLE 8 b - Regression of Heart Rate and Heat Production During Feeding.

Trial 1

Sheep	Regression*	S.E. of Regression coefficient
DC	$Y = 0.68 X - 9.2$	$\pm 0.139$
DY	$Y = 0.88 X - 47.0$	$\pm 0.259$
SY	$Y = 0.60 X - 7.3$	$\pm 0.244$
GY	$Y = 0.58 X - 1.2$	$\pm 0.188$
HY	$Y = 0.78 X + 37.8$	$\pm 0.392$
MEAN	$Y = 0.68 X - 1.6$	$\pm 0.215$

Trial 2

DC	$Y = 0.62 X - 11.8$	$\pm 0.080$
DY	$Y = 0.47 X - 7.1$	$\pm 0.261$
SY	$Y = 0.58 X - 6.1$	$\pm 0.085$
GY	$Y = 1.32 X - 52.7$	$\pm 0.251$
HY	$Y = 0.71 X - 31.4$	$\pm 0.165$
MEAN	$Y = 0.69 X - 4.0$	$\pm 0.177$

Y = Heart Rate (beats/min.)

X = Heat production (kcal/hr.)



Table 9

Regression of Heart Rate and Heat Production During Acute Cold Stress

Sheep	Regression	S.E. of regression coefficient
C <sub>1</sub>	$Y = 0.81 X - 110.9$	$\pm 0.302$
C <sub>2</sub>	$Y = 0.49 X + 42.2$	$\pm 0.381$
C <sub>3</sub>	$Y = 0.72 X - 36.6$	$\pm 0.107$
C <sub>4</sub>	$Y = 0.45 X + 49.3$	$\pm 0.166$
MEAN	$Y = 0.65 X - 22.4$	$\pm 0.159$
O <sub>1</sub>	$Y = 1.42 X - 95.4$	$\pm 0.356$
O <sub>2</sub>	$Y = 1.05 X - 92.5$	$\pm 0.174$
O <sub>3</sub>	$Y = 0.41 X + 100.7$	$\pm 0.265$
O <sub>4</sub>	$Y = 0.41 X + 69.0$	$\pm 0.087$
MEAN	$Y = 0.67 X + 3.8$	$\pm 0.153$
I <sub>1</sub>	$Y = 0.79 X - 56.2$	$\pm 0.171$
I <sub>2</sub>	$Y = 0.61 X - 69.6$	$\pm 0.175$
I <sub>3</sub>	$Y = 0.18 X + 147.6$	$\pm 0.132$
I <sub>4</sub>	$Y = 0.28 X + 105.6$	$\pm 0.054$
MEAN	$Y = 0.54 X + 5.4$	$\pm 0.049$

Y = heart rate (beats/min)

X = heat production (kcal/hr)



trials. It was felt that the activity of heart rate as a function of metabolic rate was more physiological than the comparison by Webster (1967). Thus heat production was stated as the independent variable and heart rate as the dependent variable.

Linear regression for the sheep in severe cold is shown in Table 9. These values represent heat production up to 433 kcal/0.75kg/24hr and heart rates up to 260 beats/min. The greatest amount of variation again is between animals. Mean regression values were determined for all animals in the environmental group for each time period. The control and outdoor sheep have similar slopes (0.65 and 0.67) respectively while the indoor sheep have a slope of 0.54. The mean b values for these sheep at severe cold stress are similar to the mean b values for eating (Table 8). The relationship between heat production and heart rate was similar to that suggested by Webster (1967). The error terms in the present experiments are greater because far less values have been included in each regression. The present data, however, does suggest that these relationships exist beyond the limits of energy expenditure suggested by Webster to near maximal levels of heat production.

#### Autonomic Control of Heart Rate

It has already been shown that heart rate can increase during exercise in man (Cumming and Carr, 1966) and dogs (Donald and Shephard, 1963), following surgical sympathectomy of the heart or pharmacological blockade with propranolol. Part of this increase in heart rate during exercise is undoubtedly due to reduction in





vagal tone (Robinson et al., 1966). Donald and Samueloff (1966) have suggested, however, that autonomic control cannot entirely account for cardioacceleration during increased work load. The present results tend to confirm this suggestion.

Propranolol totally inhibited direct stimulation of sympathetic nerves to the heart, which confirms that sympathetic control of the heart is mediated entirely by beta-adrenergic receptors. Atropine and propranolol together effectively isolated the heart from all autonomic control. Pharmacological cardiac denervation produced in this way is not only simpler than surgical denervation but more effective since surgical sympathectomy does not inhibit the excitatory effects of catecholamines from the adrenal medulla or from sympathetic nerves not directly supplying the heart. The use of atropine, however, does present some problems of side effects, in particular those which depress the mechanical and chemical processes of digestion and thus appetite. During naturally occurring environmental stimuli, the effects of propranolol on heart rate were variable. During mild cold exposure propranolol markedly inhibited cardio-acceleration particularly during the early phase. When shorn sheep were exposed to extreme cold stress some cardiac acceleration persisted after total beta-adrenergic blockade. Some of this increase undoubtedly resulted from a decrease in vagal tone.

Beta-adrenergic blockade had little effect on heart rate during eating when heart rate rose to about 100beats/min. However, total autonomic blockade in normal animals also increased resting heart rate from 60beats/min. to 100beats/min. a value similar to



that achieved during eating. There may be no need to seek any explanation other than decreased vagal tone to account for the increase in heart rate that accompanied eating in these trials. However, recent experiments have shown that when sheep are allowed to feed while exposed to moderate cold stress heart rate can increase to more than 200 beats/min. This lends support to the suggestion that factors other than autonomic are involved in the control of heart rate.

#### Sympathetic Control of Energy Metabolism

In many species adaptation to cold stress involves a transition from shivering to non-shivering thermogenesis as a first line defense against cold (Sellar, Scott and Thomas, 1954). Greater sympathetic neural control of metabolism is demonstrated with this change to non-shivering thermogenesis (Hsieh, Carlson and Gray, 1957). Table 5 shows results of cold-acclimated sheep which were capable of producing more heat than the control sheep in similar cold stress. Beta-adrenergic blockade resulted in a 12% inhibition of maximal heat production in the cold-acclimated sheep compared to 6% inhibition in the warm-acclimated sheep. Although the sympathetic control of metabolism was small, it appears that it did play some part in the metabolic response of cold-acclimated sheep to extreme cold stress.

Winter-acclimatized sheep did not increase heat production to levels similar to the cold-acclimated sheep. However, rectal temperature decreased at a greater rate compared to the latter animals. Winter-acclimatized sheep thus tended to decrease body



temperature more and increase heat production less than cold-acclimated animals. In other words during severe cold stress these animals exhibited heterothermic tendencies. This has adaptive significance with regard to conservations of energy during brief periods of cold exposure. At this level of heat production the winter-acclimatized sheep did not require increased heat production through sympathetic pathways.

In order for agriculture to maintain "dynamic homeostasis" with increasing population the efficiency of food production must be increased in developed areas, and eventually food must be produced in undeveloped areas. Studies based on increasing animal production for eventual food consumption aid in supporting both of the above avenues.

A greater understanding of mechanisms of physiological control of the animal not only aid the producer to increase production but also may support other fields of endeavor, whose relevance to the main topic may not be immediately apparent.

The present study concerned the possible sympathetic controls of energy metabolism and heart rate during eating and during cold stress in sheep.

The information obtained with regard to control of energy metabolism has immediate relevance to the problem of feed efficiency in ruminants exposed to natural environments particularly those which exist in Western Canada. The studies on the control of the normal and isolated heart acquire new importance in clinical medicine as cardiologists take great steps forward in their approach to the preservation of human life.



## GENERAL SUMMARY

Experiments were performed to investigate the beta-adrenergic control of energy metabolism and heart rate in the sheep during their responses to the naturally occurring stimuli of cold stress and of eating. The responses of the heart were also studied after total pharmacological denervation. A summary of the results obtained is presented below.

1. Beta adrenergic blockade with propranolol at 0.25, 0.5 and 1.0 mg/kg. inhibited sympathetic induced cardioacceleration for at least two hours. Following propranolol infusion resting heart rate increased by about 20 beats/min. This is contrary to the results obtained for dogs and for man.

2. Propranolol inhibited cardioacceleration in sheep during mild cold stress but not during eating. During severe cold stress heart rate did increase from 98 to 118 beats/min after propranolol treatment. The absolute values obtained were, however, only about half those recorded in the untreated animals exposed to severe cold.

3. Atropine at .05 mg/kg effectively blocked vagal control of heart rate. Following total pharmacological cardiac denervation with atropine and propranolol resting heart rate increased from 60 to 100 beats/min. This indicates the extent to which resting heart rate in the intact sheep is reduced by tonic vagal impulses.

4. Total pharmacological blockade did not significantly alter the resting heart rate of thyroidectomized sheep which remained at about 75 beats/min. This suggests that thyroid







hormone is important in the regulation of intrinsic heart rate in the sheep.

5. Following total pharmacological blockade, the heart rate of intact and thyroidectomized sheep did not alter significantly during eating.

6. During exposure of shorn sheep to  $-30^{\circ}\text{C}$ , cold-acclimated animals increased heat production to eight times their estimated fasting level of metabolism. Maximal heat production was 25% greater than that of the warm-acclimated and 32% greater than that of the winter-acclimatized sheep exposed to a similar air temperature. The winter acclimatized sheep appeared not to increase heat production to maximal levels but rather to permit some body cooling.

7. Propranolol reduced maximal heat production in the cold-acclimated sheep by 12% and in the warm-acclimated sheep by 6%. Propranolol had no effect on metabolic rate in the winter-acclimatized sheep at  $-30^{\circ}\text{C}$ . This supports the conclusion that these sheep did not display maximal heat production at  $-30^{\circ}\text{C}$ .



BIBLIOGRAPHY

- Adolph, E.F. 1961. Early concepts of physiological regulations  
Physiol. Rev. 41:737-770.
- Agricultural Research Council. 1965. The Nutrient Requirements of  
Farm Livestock. No. 2 Ruminants. Technical Reviews and  
Summaries.
- Ahlquist, R.P. 1948. A study of the adrenotropic receptors. Am.  
J. Physiol., 153:586-600.
- Ahlquist, R.P. 1967. Development of the concept of alpha and beta  
adrenotropic receptors. Ann. N.Y. Acad. Sci. 139:549-552.
- Alexander, G. 1962. Temperature regulation in the new-born lamb.  
V. Summit metabolism. Aust. J. Agric. Res. 13:100-121.
- Andersson, B., L. Ekman, B. Hokfelt, M. Jobin, K. Olsson and D.  
Robertshaw. 1967. Studies of the importance of the thyroid  
and the sympathetic system in the defence to cold of the goat.  
Acta Physiol. Scand. 69:111-118.
- Antonis, A., M.L. Clark, R.L. Hodge, M. Molony, and T.R.E. Pilkington.  
1967. Receptor mechanisms in the hyperglycemic response  
to adrenaline in man. Lancet 11:1135-1137.
- Ariens, E.J. 1967. The structure activity relationships of beta  
adrenergic drugs and beta adrenergic blocking drugs. Ann. N.  
Y. Acad. Sci. 139:606-631.
- Arnold, B. 1962. Activity of sheep at pasture. J. Br. Grassld.  
Soc. 17:41-62.
- Ashkar, E., J.J. Stevens and B.A. Houssay. 1968. Role of the  
sympathico-adrenal system in the hemodynamic response to  
exercise in dogs. Am. J. Physiol. 214:22-27.
- Bigelow, W.G. and S. Sidlofsky. 1961. Hormones in hypothermia.  
Brit. Med. Bull. 17:56-60.
- Bing, R.J. 1965. Cardiac metabolism. Physiol. Rev. 45:171-213.
- Black, J.W., A.F. Crowther, R.G. Shanks, L.A. Smith, and A.C.  
Dornhorst. 1964. A new adrenergic beta-receptor antagonist.  
Lancet (1):1080-1081.
- Black, J.W., W.A.M. Duncan and R.G. Shanks. 1965. Comparison of  
some properties of pronethalol and propranolol. Brit. J.  
Pharmacol. 25:577-591.



- Blaxter, K.L. 1943. The normal variation in the heart rate of dairy cows. *Vet. J.* 99:2-4.
- Blaxter, K.L. 1948. Severe experimental hyperthyroidism in the ruminant. II. Physiological effects. *J. Agric. Sci.* 38:20-27.
- Blaxter, K.L. 1962. The fasting metabolism of adult wether sheep. *Brit. J. Nutr.* 16:615-626.
- Blaxter, K.L. 1967. The energy metabolism of ruminants. London: Hutchinson & Co. (Publishers) Ltd.
- Blaxter, K.L., N. McC. Graham, F.W. Wainman and D.G. Armstrong. 1959. Environmental temperature, energy metabolism and heat regulation in sheep. II. The partition of heat losses in closely clipped sheep. *J. Agric. Sci., Camb.* 52:25-40.
- Blaxter, K.L. and J.P. Joyce. 1963. The accuracy and ease with which measurements of respiratory metabolism can be made with tracheostomized sheep. *Brit. J. Nutr.* 17:523-537.
- Bligh, J. 1966. The thermosensitivity of the hypothalamus and thermoregulation in mammals. *Biol. Rev. Cambridge Phil. Soc.* 41:317-367.
- Blinks, J.R. 1967. Evaluation of the cardiac effects of several beta adrenergic blocking agents. *Ann. N.Y. Acad. Sci.* 139: 673-685.
- Braunwald, E. 1966. Heart. *Ann. Rev. Physiol.* 28:227-266.
- Brick, I. and K.J. Hutchison. 1966. The effect of  $\beta$ -receptor antagonist (propranolol) on the response of the isolated perfused rats' heart to noradrenaline. *J. Physiol.* 183: 37-38.
- Brouwer, E. 1965. Publ. European Assoc. Animal Prod. No. 11, Academic Press, London, p. 411.
- Byerby, T.C. 1966. The role of animal agriculture in meeting food needs. *Proceedings (50 th.)*. Agric. Res. Inst. Washington, D.C.
- Canadian Cold Physiology Conference (9th). 1967. Cold Thermogenesis. University of Alberta, Edmonton.
- Cannon, W.B. 1929. Organization for physiological homeostasis. *Physiol. Rev.* 9:399-431.



- Cannon, W.B. and Z.M. Bacq. 1931. Studies on the conditions of activity in endocrine organs. XXVI. A hormone produced by sympathetic action on smooth muscle. Am. J. Physiol. 96: 392-412.
- Carlson, L.D. 1960. Non-shivering thermogenesis and its endocrine control. Fed. Proc. 19:25-30.
- Carlson, L.D. 1966. The role of catecholamines in cold adaptation. Pharmacol. Rev. 18:291-301.
- Carlson, L.D., H.L. Burns, T.H. Holmes and P.P. Webb. 1953. Adaptive changes during exposure to cold. J. Appl. Physiol. 5: 672-676.
- Chapman, C.B. and J.H. Mitchell. 1965. Starling on the Heart. Dawsons of Pall Mall, London.
- Chatonnet, J. and Y. Minaire. 1966. Comparison of energy expenditure during exercise and cold exposure. Fed. Proc. 25:1348-1350.
- Clapperton, J.L. 1964. The energy metabolism of sheep walking on the level and on gradients. Brit. J. Nutr. 18:47-54.
- Cronin, R.F.P. 1967. Hemodynamic and metabolic effects of  $\beta$ -adrenergic blockade in exercising dogs. J. Appl. Physiol. 22(2): 211-216.
- Cumming, G.R. and W. Carr. 1966. Hemodynamic response to exercise after propranolol in normal subjects. Can. J. Physiol. Pharmacol. 44:465-474.
- Cumming, G.R. and W. Carr. 1967. Hemodynamic response to exercise after beta-adrenergic and parasympathetic blockade. Can. J. Physiol. Pharmacol. 45:813-819.
- Dale, H.H. 1906. On some physiological actions of ergot. J. Physiol. 34:163-206.
- Dawkins, M.J.R. and J.W. Scopes. 1965. Non-shivering thermogenesis and brown adipose tissue in the human new-born infant. Nature: 206:201-203.
- Donald, D.E. and S. Milburn. 1968. Role of cardiac autonomic nerves and circulating norepinephrine in maximal exercise. Fed. Proc. 27(2):231.







- Donald, D.E. and S.L. Samueloff. 1966. Exercise tachycardia not due to blood borne agents in canine cardiac denervation. *Am. J. Physiol.* 211(3):703-711.
- Donald, D.E. and J.T. Shephard. 1963. Response to exercise in dogs with cardiac denervations. *Am. J. Physiol.* 205(2):393-400.
- Donald, D.E. and J.T. Shephard. 1964. Sustained capacity for exercise in dogs after complete cardiac denervation. *Am. J. Cardio.* 14:853-860.
- Donhoffer, S.Z., F. Sárdy and G.Y. Szegvari. 1964. Brown adipose tissue and thermoregulatory heat production in the rat. *Nature* 203:765-766.
- Epstein, S.E., Brian Robinson, R.L. Kahlor and E. Braunwald. 1965. Effects of beta adrenergic blockade on the cardiac response to maximal and submaximal exercise in man. *J. Clin. Invest.* 44:1745-1753.
- Ezdinli, E.Z., R. Javid, G. Owens and J.E. Sokal. 1968. Effect of high spinal cord section on epinephrine hyperglycemia. *Am. J. Physiol.* 214:1019-1024.
- Furchgott, R.F. 1967. The pharmacological differentiation of adrenergic receptors. *Ann. N.Y. Acad. Sci.* 139:553-570.
- Gasser, H.S. and W.J. Meek. 1914. A study of the mechanism by which muscular exercise produces acceleration of the heart. *Am. J. Physiol.* 34:48-71.
- Graham, N.McC. 1964. Energy cost of feeding activities and energy expenditure of grazing sheep. *Aust. J. Agric. Res.* 15: 969-973.
- Glaser, E.M. 1950. Acclimatization to heat and cold. *J. Physiol.* 110:330-337.
- Glick, G., S.E. Epstein, A.S. Wechsler and E. Braunwald. 1967. Physiological differences between the effects of neuronally released and blood borne norepinephrine on beta-adrenergic receptors in the arterial bed of the dog. *Circ. Res.* 21: 217-227.
- Glickman, N., H.H. Mitchell, R.W. Keeton and E.A. Lambert. 1967. Shivering and heat production in man exposed to intense cold. *J. Appl. Physiol.* 22(1):1-8.



- Hardy, J.D. 1961. Physiology of temperature regulation. *Physiol. Rev.* 41:521-606.
- Hart, J.S. 1961. Physiological effects of continued cold on animals and man. *Brit. Med. Bull.* 17:19-24.
- Hayward, J.S. 1967. Review: Sites of nonshivering heat production. Session I. Ninth Canadian Cold Physiology Conference. University of Alberta.
- Hayward, J.S., C.D. Lyman and C.P. Taylor. 1965. The possible role of brown fat as a source of heat during arousal from hibernation. *Ann. N.Y. Acad. Sci.* 131:441-446.
- Heim, T. and D. Hull. 1966. The effect of propranolol on the calorigenic response in brown adipose tissue of new-born rabbits to catecholamines, glucagon, corticotrophin and cold exposure. *J. Physio.* 187:271-283.
- Hemingway, A. 1963. Shivering. *Physiol. Rev.* 43:397-422.
- Hemingway, A. and W.M. Price. 1964. The calorigenic action of catecholamines in warm acclimated and cold acclimated non-shivering cats. *Intern. J. Neuropharmacol.* 3:495-503.
- Hemingway, A. and W.M. Price. 1968. The autonomic nervous system and regulation of body temperature. *Anesthesiology* 29: 693-701.
- Heroux, O. 1966. Metabolic adjustments to low temperatures in New Zealand white rabbits. *Can. J. Physiol. Pharmacol.* 45: 451-461.
- Himms-Hagen, J. 1965. Lipid metabolism in warm-acclimated and cold-acclimated rats exposed to cold. *Can. J. Physiol. Pharmacol.* 43:379-403.
- Himms-Hagen, J. 1967. Sympathetic regulation of metabolism. *Pharmacol. Rev.* 19:367-461.
- Hoffman, B.F. 1962. Origin of the heart beat in Cardiovascular Functions. Ed. Suisana, A.A.:2-31, 2-41. McGraw-Hill Book Company, Inc. Toronto.
- Hsieh, A.C.L. and L.D. Carlson. 1957. The role of adrenaline and noradrenaline in chemical regulation of heat production. *Am. J. Physiol.* 190:243-246.
- Hsieh, A.C.L., L.D. Carlson and G. Gray. 1957. Role of the sympathetic nervous system in the control of chemical regulation of heat production. *Am. J. Physiol.* 190:247-251.



- Hsieh, A.C.L., C.W. Pun, K.M. Li and K.W. Ti. 1966. Circulatory and metabolic effects of noradrenaline in cold-adapted rats. Fed. Proc. 25:1205-1209.
- Issekutz, B., Jr., H.T. Miller and K. Rodahl. 1966. Lipid and carbohydrate metabolism during exercise. Fed. Proc 25:1415-1420.
- Jansky, L. 1966. Body organ thermogenesis of the rat during exposure to cold and at maximal metabolic rate. Fed. Proc. 25: 1297-1302.
- Johnson, G.L. 1968. Influence of thyroxine on vascular reactivity. Fed. Proc. 27(1 ):536.
- Johnson, G.E., E. Schönbaum and E.A. Sellers. 1966. Cold exposure: pharmacological investigation of the compensatory mechanisms in the maintenance of normothermia. Fed. Proc. 25:1216-1219.
- Jose, A.D. 1966. Effect of combined sympathetic and parasympathetic blockade on heart rate and cardiac function in man. Am. J. Cardio. 18:476-478.
- Jose, A.D. and F. Stitt. 1967. Cardiac function after combined beta-adrenergic and cholinergic blockade. Relationships of intrinsic rate to contractile force of heart in dogs. Circ. Res. 21:Suppl. 3:231-242.
- Joyce, J.P. and K.L. Blaxter. 1964. The effects of air movement, air temperature and infrared radiation on the energy requirements of sheep. Brit. J. Nutr. 18:5-27.
- Kleiber, Max. 1961. The Fire of Life: An Introduction to Animal Energetics. New York: John Wiley and Sons, Inc.
- Kopin, I.J. 1966. Biochemical aspects of release of norepinephrine and other amines from sympathetic nerve endings. Pharmacol. Rev. 18:513-523.
- Kunos, G. and M. Szentinanyi. 1968. Evidence favoring the existence of a single adrenergic receptor. Nature 217:1077-1078.
- Lange, G., H.H. Lu, A. Chang and C. Brooks. 1966. Effect of stretch on the isolated cat sino-arterial node. Am. J. Physiol. 211: 1192-1196.
- Ledsome, J.R., R.J. Linden and J. Norman. 1965. The use of sympathetic beta-receptor blocking agents in the investigation of reflex changes in heart rate. Brit. J. Pharmacol. 24:781-788.





- Loewi, O. 1921. Über humorale Übertragung der Hergnervenerregung. Pflüger. Arch. ges. Physiol. 189:239-252.
- McDevitt, D.G., R.G. Shanks, D.R. Hadden, D.A.D. Montgomery and J.A. Weaver. 1968. The role of the thyroid in the control of heart rate. Lancet 1:998-1000.
- McInermy, T.K., D.P. Gilmour and J.R. Blinks. 1965. Comparison of effects of propranolol and other cardiac adrenergic blocking agents on inotropic and chronotropic actions of catecholamines. Fed. Proc. 24:712.
- Malhotra, M.S., J. Sen Gupta and R.M. Rai. 1963. Pulse count as a measure of energy expenditure. J. Appl. Physiol. 18(5):994-996.
- Margolius, H.S. and T.E. Gaffrey. 1965. The effects of injected norepinephrine and sympathetic nerve stimulation in hypothyroid and hyperthyroid dogs. J. Pharmac. exp. Ther. 149:329-335.
- Mayer, S.E., M. DeV. Cotten and N.C. Moran. 1963. Dissociation of the augmentation of cardiac contractile force from the activation of myocardial phosphorylase by catecholamines. J. Pharm. exp. Ther. 139:275-282.
- Mayer, S.E., B.J. Williams and J.M. Smith. 1967. Adrenergic mechanisms in cardiac glycogen metabolism. Ann. N.Y. Acad. Sci. 139:686-702.
- Mitchell, H.H. 1928. Livestock investigations. 1927-1928. Ann. Rep. Univ. Ill. Agric. Exp. Stn., p. 155.
- Moore, R.E. and M.C. Underwood. 1963. The thermogenic effects on noradrenaline in new-born and infant kittens and other small mammals. A possible hormonal mechanism in the control of heat production. J. Physiol. 168:290-317.
- Moran, N.C. and M.E. Perkins. 1958. Adrenergic blockade of the mammalian heart by a dichloro analogue of isoproterenol. J. Pharm. Exp. Ther. 124:223-237.
- Muir, C.G., D.A. Chamberlain and D.T. Pedoe. 1964. Effects of beta-sympathetic blockade on non-esterified fatty acid and carbohydrate metabolism at rest and during exercise. Lancet: 2: 930-932.
- Nakano, J. and T. Kusakari. 1965. Competitive antagonisms between isoproterenol and a new  $\beta$ -receptor adrenergic blocking agent propranolol. Proc. Soc. Exp. Biol. Med. 119(2):350-352.





- Namm, D.H. and S.E. Mayer. 1968. Effects of epinephrine on cardiac 3' 5'-AMP, phosphorylase kinase and phosphorylase. *Mol. Pharmacol.* 4 (1): 61-69.
- Oliver, G. and E.A. Schafer. 1895. On the physiological action of extracts of pituitary body and certain other glandular organs. *J. Physiol.* 18: 230-276.
- Pathak, C.L. 1966. The fallacy of the Bainbridge reflex. *Am. Heart J.* 72: 577-581.
- Paul, Paula and Bela Issekutz, Jr. 1967. Role of extramuscular energy sources in the metabolism of the exercising dog. *J. Appl. Physiol.* 22 (4): 615-622.
- Pilkington, T.R.E., R.D. Lowe, B.F. Robinson and E. Titterington. 1962. Effect of adrenergic blockade on glucose and fatty-acid mobilization in man. *Lancet* 2: 316-317.
- Pearson, E.S. and H.O. Hartley, Ed. 1962. Biometrika Tables for Statisticians. Vol. 1, Cambridge University Press, London.
- Powell, C.E. and I.H. Slater. 1958. Blocking of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. *J. Pharm. exp. Ther.* 122: 480-488.
- Rall, T.W. and E.W. Sutherland. 1958. Formation of a cyclic adenine ribonucleotide by tissue particles. *J. Biol. Chem.* 232: 1065-1076.
- Rall, T.W. and E.W. Sutherland. 1962. Adenyl cyclase. II. The enzymatically catalyzed formation of adenosine 3',5'-phosphate and inorganic pyrophosphate from adenosine triphosphate. *J. Biol. Chem.* 237: 1228-1232.
- Read, J.M. 1924. Basal pulse rate and pulse pressure changes accompanying variations in the basal metabolic rate. *Arch. intern. Med.* 34: 553-572.
- Robinson, B.F., S.E. Epstein, G.D. Beiser and E. Braunwald. 1966. Control of heart rate by the autonomic nervous system. *Circ. Res.* 19: 400-411.
- Robinson, B.F., R.L. Kahler, S.E. Epstein and E. Braunwald. 1965. Effects of beta-adrenergic blockade in man on the hemodynamic response to maximal exercise. *Fed. Proc.* 24: 590.
- Robison, G.A., R.W. Butcher and E.W. Sutherland. 1967. Adenyl cyclase as an adrenergic receptor. *Ann. N.Y. Acad. Sci.* 139: 703-723.
- Schönbaum, E., G.E. Johnson, E.A. Sellers and M.J. Gill. 1966. Adrenergic  $\beta$ -receptors and non-shivering thermogenesis. *Nature* 210: 426.
- Sellers, E.A., J.W. Scott and N. Thomas. 1954. Electrical activity of skeletal muscle of normal and acclimatized rats on exposure to cold. *Am. J. Physiol.* 177: 372-376.



- Shanks, R.G. 1966. The effect of propranolol on the cardiovascular responses to isoprenaline, adrenalin, noradrenalin in the anaesthetized dog. Brit. J. Pharm. Chem. 26 (2) 322-333.
- Slee, J. and A.R. Sykes. 1967. Acclimatization of Scottish blackface sheep to cold. 1. Rectal temperature responses. Anim. Prod. 9 (3): 333-347.
- Smillie, K.W. 1968. Statpack I: An APL Statistical Package. Pub. 9, Dept. Comp. Sci. University of Alberta.
- Smith, R.E. and J.C. Roberts. 1964. Thermogenesis of brown adipose tissue in cold acclimated rats. Am. J. Physiol. 206: 143-148.
- Sonnenblick, E.H., E. Braunwald, J.F. Williams and G. Glick. 1965. Effects of exercise on myocardial force-velocity relations in intact, unanesthetized man: Relative roles of changes in heart rate, sympathetic activity and ventricular dimensions. J. Clin. Invest. 44 (12): 2051-2062.
- Stecher, P.G. ed. 1960. The Merck Index of Chemicals and Drugs. Merck and Co. Inc. Ralway, N.J. U.S.A., 7th Ed.
- Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc. New York.
- Steiner, G., E. Schonbaum, G.E. Johnson and E.A. Sellers. 1968. Lipid metabolism: Effects of immunosympathectomy and acclimation to cold. Can. J. Physiol. Pharmacol. 46: 453-461.
- Sutherland, E.W. and G.A. Robison. 1966. The role of cyclic 3',5' AMP in responses to catecholamines and other hormones. Pharmacol. Rev. 18: 145-161.
- Suzutti, M., T. Tonone, S. Matsuzaki and K. Yamamoto. 1967. Initial response of human thyroid, adrenal cortex and adrenal medulla to acute cold exposure. Can. J. Physiol. Pharmacol. 45: 423-432.
- Swift, R.W. 1932. The effects of low environmental temperature upon metabolism II. The influence of shivering, subcutaneous fat, and skin temperature on heat production. J. Nutr. 5: 227-249.
- Sykes, A.R. and J. Slee. 1968. Acclimatization of Scottish blackface sheep to cold. 2. Skin temperature, heart rate, re piration rate, shivering intensity and skinfold thickness. Anim. Prod. 10(1): 17-35.
- Trendelenburg, U. 1966. I. Mechanisms of supersensitivity and sub-sensitivity to sympathomimetic amines. Pharmacol. Rev. 18: 629-640.
- Waites, G.M.H. 1957. The course of the efferent cardiac nerves of the sheep. J. Physiol. 139: 417-433.
- Webster, A.J.F. 1966a. Studies in the Energy Metabolism of Ruminants. Ph.D. Thesis, University of Glasgow.



- Webster, A.J.F. 1966b. The establishment of thermal equilibrium in sheep exposed to cold environments. *Res. Vet. Sci.* 7: 454-465.
- Webster, A.J.F. 1967. Continuous measurement of heart rate as an indicator of the energy expenditure of sheep. *Brit. J. Nutr.* 21: 769-785.
- Webster, A.J.F. and K.L. Blaxter. 1966. The thermal regulation of two breeds of sheep exposed to air temperature below freezing point. *Res. Vet. Sci.* 7: 466-479.
- Webster, A.J.F. and F.L. Hays. 1968. Effects of beta-adrenergic blockade on the heart rate and energy expenditure of sheep during feeding and during acute cold exposure. *Can. J. Physiol. Pharmacol.* 46: 577-583.
- Webster, A.J.F. and A.M. Hicks. 1968a. Respiration apparatus for the determination of the energy expenditure of livestock in cold environment. *Can. J. An. Sci.* 48: 89-92.
- Webster, A.J.F. and A.M. Hicks. 1968b. Physiological responses of sheep to winter conditions in Alberta. *Feeders' Day Report, Dept. of An. Sci., University of Alberta.* 38-40.
- Webster, A.J.F. and C. Park. 1967. The effect of jute coats on the heat losses of two breeds of sheep exposed to different environments. *Anim. Prod.* 9: 483-490.
- Webster, A.J.F. and D. Valks. 1966. The energy cost of standing in sheep. *Proc. Nutr. Soc.* 25, xxii.
- Williamson, J.R. 1966. Kinetic studies of epinephrine effects in the perfused rat heart. *Pharm. Rev.* 18: 205-210.
- Wodzicka, M. 1958. Studies of the thickness and chemical composition of the skin of sheep. III. Effect of Shearing. *N.Z. J. Agric. Res.* 1: 601-606.
- Young, B.A. 1966. Energy expenditure and respiratory activity of sheep during feeding. *Aust. J. Agric. Res.* 17: 355-362.
- Zotterman, Y. 1953. Special senses: Thermal receptors. *Ann. Rev. Physiol.* 15: 357-372.





# APPENDIX 1

TABLE 10 Heart Rate during Cold Exposure (beats/min.)

	Time (hours)							
	0.5	1	1.5	2	2.5	3	3.5	4
Warm control	Warm				Cool			
	72+13	71+13	68+11	65+11	65+11	61+9	61+8	61+11
Warm Prop (0.5 mg/kg)	78+25	75+20	74+13	72+14	71+13	71+14	72+14	83+17
Cold control	Cool				Cold			
	77+15	76+15	96+36	106+40	125+36	132+36	126+36	112+32
Cold Prop (0.25 mg/kg)	81+12	74+8	83+23	85+27	87+25	81+20	87+24	91+31
Cold Prop (0.5 mg/kg)	79+15	72+13	76+14	81+17	83+21	87+21	85+21	88+20
Cold Prop (1.0 mg/kg)	74+14	76+17	77+23	79+25	77+25	81+24	85+26	88+30

\*S.D.

Mean heart rates for all sheep in the cold exposure trials (For an explanation see the text). These values are the average for each 30 minute period. The values in the brackets are standard deviations for all sheep.





APPENDIX 2

TABLE 11- Heat Production during Cold Exposure (kcal/hr.)

	Time (hours)									
	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
Warm control	98.4+21.3	88.6+21.3	101.5+21.4	102.5+21.3	107.2+15.8	103.9+12.1	103.8+11.7	103.8+12.9	100.0+10.3	99.6+8.1
Warm prop (0.5 mg/kg.)	109.8+13.3	111.3+15.3	106.0+29.3	108.8+21.2	103.7+23.6	101.6+22.8	101.7+16.4	98.1+16.5	94.9+16.3	95.3+16.3
Cold control	121.7+16.9	116.3+18.2	150.0+25.1	165.0+25.7	178.7+21.9	171.6+17.4	161.4+18.7	159.0+22.3	148.6+16.0	162.5+16.8
Cold prop (0.25 mg/kg)	130.5+10.7	140.7+30.3	148.0+31.0	162.4+25.5	176.4+33.0	163.7+24.8	163.5+37.7	158.4+40.6	146.6+26.1	139.6+12.9
Cold prop (0.5 mg/kg)	139.2+22.7	126.9+19.5	144.1+22.3	162.4+40.6	162.4+28.2	168.2+35.5	164.6+33.5	163.5+39.0	161.1+35.5	159.7+43.2
Cold prop (1.0 mg/kg)	116.9+23.1	131.5+10.1	130.2+11.2	137.4+5.8	134.3+4.7	139.2+13.0	151.5+25.5	141.8+33.5	141.4+33.0	128.4+25.4

\*S.D.

Mean heat production (kcal/hr.) for all sheep in the cold exposure trials calculated from O<sub>2</sub> and CO<sub>2</sub> values (For and explanation see the text). Each of the values is average heat production for each 30 minute period. The standard deviation for all animals is shown in brackets.



TABLE 12. Rectal Temperature (°C) During Cold Exposure

	Time (hours)									
	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
	Warm					Cool				
Warm control	39.5±.4*	39.5±.3	39.3±.4	39.2±.5	39.2±.5	39.2±.3	39.1±.4	38.0±.4	39.0±.4	38.9±.4
Warm prop (0.5 mg/kg)	39.6±.8	40.0±.5	40.3±.4	40.4±.5	40.4±.6	40.3±.7	40.1±.9	40.1±.1	40.1±.9	40.0±.9
	Cool					Cold				
Cold control	39.6±.2	39.5±.2	39.6±.3	39.6±.3	39.6±.3	39.5±.3	39.5±.3	39.4±.2	39.4±.2	39.4±.2
Cold prop (0.25 mg/kg)	39.9±.1	40.2±.1	40.4±.2	40.4±.3	40.4±.4	40.5±.5	40.5±.4	40.4±.4	40.2±.3	40.0±.4
Cold prop (0.5 mg/kg)	39.8±.6	39.9±.4	40.1±.3	40.1±.3	40.1±.4	39.8±.2	39.7±.3	39.7±.3	39.5±.3	39.4±.4
Cold prop (1.0 mg/kg)	39.6±.4	39.7±.3	40.0±.2	40.0±.7	40.1±.7	40.0±.8	39.7±.6	39.7±.5	39.6±.4	39.5±.4

\*S.D.

Mean rectal temperatures of sheep during the sheep in the warm and the cold with and without propranolol.



APPENDIX 4a

Mean Squares - Experiment 1

Cold Exposure

Source	d.f.	Heart rate M.S.	Heat production M.S	O <sub>2</sub> pulse M.S.	CO <sub>2</sub> pulse
1	5	11234****	32437****	22.33**	14.24*
2	4	17155****	9672***	91.22****	69.43****
3	7	348N.S.	860***	2.68N.S.	3.06N.S.
1 x 2	20	1455****	2452****	6.31****	3.17****
1 x 3	35	233****	207N.S.	0.76****	0.75****
2 x 3	28	91*	301N.S.	0.35N.S.	0.25N.S.
Error	140	52	153	0.29	0.21
Total	239				

1 Treatments of cold exposure.

2 Animals.

3 Time periods.

Probability of significant difference:

\*\*\*\*0.1%

\*\*\*0.5%

\*\*1.0%

\*5.0%



APPENDIX 4b

Mean Differences - Experiment 1  
( $P < 0.01$ )  
Cold Exposure

Duncan's new multiple range test  
(underlined values are significantly the same)

1. Heart rate

a) Treatments of cold  $S\bar{x} = 2.30$

T-Con	T-P <sub>2</sub>	C-P <sub>3</sub>	CP <sub>2</sub>	CP <sub>1</sub>	C-Con
62.96	76.66	<u>83.75</u>	85.94	<u>83.13</u>	114.01
T-Con	T-P <sub>2</sub>	(C-P <sub>3</sub> CP <sub>2</sub> CP <sub>1</sub> )*			C-Con
62.96	76.66	85.60			114.01

b) Animals  $S\bar{x} = 2.10$

DY	DC	GY	SY	HY
<u>70.45</u>	<u>76.35</u>	<u>77.34</u>	<u>83.38</u>	117.89

2. Heat production

a) Treatments of cold  $S\bar{x} = 3.11$

T-P <sub>2</sub>	T-Con	C-P <sub>3</sub>	C-P <sub>1</sub>	C-P <sub>2</sub>	C-Con
<u>101.27</u>	<u>102.79</u>	138.01	<u>157.33</u>	<u>160.75</u>	<u>162.09</u>

b) Animals  $S\bar{x} = 2.84$

GY	HY	DC	DY	SY
<u>121.65</u>	<u>126.10</u>	<u>133.78</u>	<u>151.18</u>	<u>152.50</u>

\* Mean Propranolol Values





APPENDIX 4b cont.

3. O<sub>2</sub> Pulse

a) Animals  $\bar{Sx} = 0.4$

HY	GY	DC	SY	DY
3.64	5.31	<u>5.95</u>	6.47	<u>7.29</u>

4. CO<sub>2</sub> Pulse

a) Animals  $\bar{Sx} = 0.30$

HY	GY	DC	SY	DY
3.24	5.23	<u>5.56</u>	<u>5.59</u>	<u>6.49</u>



# APPENDIX 5

TABLE 13. Preliminary Feeding Trials - Heart Rates  
beats/min

(periods of 10 min)	1	2	3	4	5	6	7	Post-feeding			
								Eating			
	Pre-feed							8	9	10	11
Control	63±12	86±21*	94±19	107±16	113±17	109±19	101±11	79±20	82±15	78±11	75±9
Saline	59±7	111±35	119±35	117±34	112±33	106±29	107±44	90±43	76±20	72±14	68±14
Prop (0.25 mg/kg)	73±15	83±12	89±18	98±13	96±10	103±16	92±13	85±14	79±12	77±10	77±13
Prop (0.5 mg/kg)	73±15	87±18	96±15	99±10	102±7	102±19	93±19	89±18	82±13	83±17	84±16
Prop (1.0 mg/kg)	70±12	86±10	97±14	102±12	103±15	97±10	103±9	78±10	79±9	79±12	76±10

\* S.D.

Mean heart rates for preliminary feeding trials in individual holding crates with a control, a saline infusion and three levels of propranolol (0.25, 0.5 and 1.0 mg/kg).



APPENDIX 6a

Mean Squares - Experiment 1

Feeding Trials (preliminary)

Heart Rate

---

Source	d.f.	M.S.
1	4	398.0 N.S.
2	10	4406.2****
3	4	3983.8****
Error	256	210.3
Total	274	

---

1. Treatments during feeding

2. Times

3. Animals

\*\*\*\* P < 0.001



APPENDIX 6b

Mean Differences - Experiment 1  
( $P < 0.01$ )  
Feeding Trials (preliminary)

Duncan's New Multiple Range Test  
(underlined values are significantly the same)

Heart Rate

a) Times during the trials  $S\bar{x} = 2.90$

1	11	10	9	8	2	3	7	6	5	4
69	79	78	80	84	<u>92</u>	<u>99</u>	<u>99</u>	<u>103</u>	<u>105</u>	<u>105</u>

b) Animals  $S\bar{x} = 1.96$

DY	SY	GY	HY	DC
<u>80.4</u>	<u>81.0</u>	<u>93.3</u>	<u>95.5</u>	<u>98.7</u>





APPENDIX 7

TABLE 14. Heart Rates During Feeding  
(beats/min)

	Time (hours) eating				
	0.5	1	1.5	2	2.5
Control 1	60 $\pm$ 20*	99 $\pm$ 20	123 $\pm$ 18	106 $\pm$ 25	84 $\pm$ 32
Control 2	55 $\pm$ 20	87 $\pm$ 15	113 $\pm$ 24	90 $\pm$ 21	75 $\pm$ 24
Mean	57 $\pm$ 21	91 $\pm$ 22	118 $\pm$ 25	98 $\pm$ 26	80 $\pm$ 33
Propranolol 1	60 $\pm$ 15	80 $\pm$ 10	98 $\pm$ 20	97 $\pm$ 26	86 $\pm$ 25
Propranolol 2	62 $\pm$ 16	82 $\pm$ 8	90 $\pm$ 8	85 $\pm$ 20	83 $\pm$ 16
Mean	61 $\pm$ 14	81 $\pm$ 9	94 $\pm$ 14	91 $\pm$ 19	85 $\pm$ 20

TABLE 15 . Heat Production During Feeding  
(kcal/hr)

	Time (hours) eating				
	0.5	1	1.5	2	2.5
Control 1	93.8 $\pm$ 9.7*	150.9 $\pm$ 26.0	164.0 $\pm$ 22.8	131.5 $\pm$ 12.6	121.0 $\pm$ 19.7
Control 2	94.4 $\pm$ 14.3	163.3 $\pm$ 23.7	170.1 $\pm$ 31.0	124.8 $\pm$ 17.9	120.8 $\pm$ 11.9
Mean	94.1 $\pm$ 14.4	157.1 $\pm$ 23.8	167.1 $\pm$ 31.1	128.1 $\pm$ 17.9	120.9 $\pm$ 19.8
Propranolol 1	105.9 $\pm$ 12.6	169.7 $\pm$ 6.0	181.1 $\pm$ 15.7	141.6 $\pm$ 13.8	135.9 $\pm$ 20.4
Propranolol 2	99.4 $\pm$ 27.9	153.5 $\pm$ 46.4	156.5 $\pm$ 46.4	125.3 $\pm$ 32.8	123.8 $\pm$ 32.7
Mean	102.7 $\pm$ 27.9	161.6 $\pm$ 46.4	168.8 $\pm$ 41.2	133.5 $\pm$ 32.9	129.9 $\pm$ 32.8

\*S.D.



APPENDIX 8a

Mean Squares - Experiment 1

Feeding Trials

---

Source	d.f.	Heart rate M.S.	Heat production M.S.
1	3	7743**	1260.3**
2	4	6028*****	15612.0*****
3	4	5542*****	5329.0*****
Error	88	136	309.6
Total	99		

---

1 Treatments during feeding.

2 Times during feeding.

3 Animals.

Probability of significant difference:

\*\*\*\*0.1%

\*\*\*0.5%

\*\*1.0%

\*5.0%



APPENDIX 8b

Mean Differences - Experiment 1  
Feeding Trials  
( $P < 0.01$ )

Duncan's new multiple range test  
(underlined values are significantly the same)

1. Heart Rate

- a) Times during exposure  $S\bar{x} = 2.61$   
(see Appendix 7 )

a	e	b	d	c
59.1	82.1	86.4	94.3	106.0

- b) Animals  $S\bar{x} = 2.61$

SY	DC	GY	DY	HY
74.4	76.1	79.2	83.6	114.7

2. Heat Production

- a) Times during exposure  $S\bar{x} = 3.93$   
(See Appendix 7 )

a	e	d	b	c
98.4	125.3	130.8	159.4	167.9

- b) Animals  $S\bar{x} = 3.93$

HY	GY	DC	SY	DY
111.4	136.6	136.6	140.2	157.1



APPENDIX 9

TABLE 15 - Mean Heart Rates of Sheep at Summit Metabolism (beats/min.)

	Time (hours)					
	0.5	1.0	1.5	2.0	2.5	3
Con-Con	131+22*	213+21	205+22	204+23	195+29	205+13
Con-Out	118+15	148+15	156+17	162+16	150+15	160+23
Con-In	159+36	207+21	225+27	235+22	227+ 8	230+12
Prop-Con	105+17	122+18	119+15	118+13	120+14	123+10
Prop-Out	88+ 4	91+ 7	101+12	98+ 8	102+15	109+19
Prop-In	98+ 5	113+14	108+13	116+16	117+16	118+17

\*S. D.

Mean heart rates for sheep subjected to extreme cold stress (-30°C). These values are average for all sheep during each 30 minute period of exposure.





# APPENDIX 10a

TABLE 16 - Heat Production (kcal/hr) of Sheep at Summit Metabolism

	Time (hours)					
	0.5	1	1.5	2	2.5	3
Con-Con	244.8+52.9*	319.4+44.8	352.5+24.7	341.2+33.2	349.5+41.9	350.3+30.2
Con-Out	182.4+23.9	197.5+16.4	221.0+27.9	235.9+41.5	235.9+32.0	231.3+24.5
Con-In	282.0+57.3	356.0+29.2	397.8+75.4	408.1+73.9	415.9+81.9	399.1+68.5
Prop-Con	222.5+62.3	292.3+37.7	320.6+39.5	309.6+45.1	327.3+44.0	331.7+19.0
Prop-Out	181.8+27.5	203.3+18.2	227.6+22.7	224.4+20.7	231.5+11.6	244.6+47.3
Prop-In	263.0+72.3	322.4+64.8	323.3+104.4	375.6+81.5	340.7+84.9	350.4+111.3

\*S.D.



APPENDIX 10b

TABLE 17 - Heat Production (kcal/m<sup>2</sup>.24hr) at Summit Metabolism

	Time (hours)					
	0.5	1	1.5	2	2.5	3
Con-Con	3767+ <u>814*</u>	4914+ <u>689</u>	5423+ <u>379</u>	5238+ <u>511</u>	5377+ <u>644</u>	5389+ <u>464</u>
Con-Out	3944+ <u>852</u>	5144+ <u>721</u>	5678+ <u>397</u>	5495+ <u>534</u>	5629+ <u>674</u>	5642+ <u>486</u>
Con-In	4603+ <u>935</u>	5813+ <u>476</u>	6495+ <u>1231</u>	6633+ <u>1206</u>	6791+ <u>1337</u>	6515+ <u>1118</u>
Prop-Con	3425+ <u>958</u>	4496+ <u>580</u>	4932+ <u>608</u>	4762+ <u>694</u>	5034+ <u>677</u>	5103+ <u>293</u>
Prop-Out	2928+ <u>442</u>	3275+ <u>293</u>	3665+ <u>365</u>	3614+ <u>334</u>	3729+ <u>186</u>	3940+ <u>762</u>
Prop-In	4294+ <u>1180</u>	5264+ <u>1057</u>	5278+ <u>1705</u>	6131+ <u>1331</u>	5562+ <u>1385</u>	5721+ <u>1817</u>

\*S.D.



APPENDIX 11

TABLE 18 - Rectal Temperatures of Sheep at Summit Metabolism (°C)

	Time (hours)						
	0	0.5	1	1.5	2	2.5	3
Con-Con	39.2+0.1*	39.4+0.4	39.4+0.5	39.2+0.4	38.8+0.6	38.6+0.6	38.4+0.5
Con-Out	39.7+0.4	40.1+0.1	39.7+0.4	39.4+0.6	39.3+0.6	39.0+0.8	38.7+0.8
Con-In	39.4+0.6	39.7+0.4	39.5+0.5	39.3+0.4	39.2+0.5	39.0+0.6	39.1+0.6
Prop-Con	39.4+0.6	39.9+0.6	40.0+0.6	39.8+0.9	39.4+0.9	39.2+0.9	39.1+0.9
Prop-Out	40.2+0.3	40.3+0.4	39.8+0.3	39.5+0.8	39.3+0.8	39.1+0.9	39.0+1.0
Prop-In	40.4+0.4	40.7+0.6	40.5+0.7	40.1+0.7	39.7+0.5	39.4+0.8	39.2+1.3

\*S.D.

Mean rectal temperatures for sheep subjected to extreme cold stress (-30°C).  
Values represent the average temperatures for each 30 minute period.



APPENDIX 12

TABLE 19 - Summit Metabolism ( $T_S$   $^{\circ}C$ )  
Skin Temperatures

	Time (hours)					
	0.5	1	1.5	2	2.5	3
Con-Con	13.1+3.0*	12.9+1.5	12.5+2.2	12.1+2.2	12.2+1.4	11.3+1.8
Con-Out	12.3+1.3	11.6+2.4	10.8+1.8	10.0+2.1	9.6+2.1	9.3+1.8
Con-In	11.9+2.2	11.0+2.7	10.2+2.4	10.0+2.4	9.2+2.1	8.8+2.4
Prop-Con	14.1+2.9	13.0+3.4	12.2+2.7	11.6+2.5	11.0+2.2	11.2+2.7
Prop-Out	15.4+5.2	14.5+4.8	13.9+5.3	13.0+5.2	12.4+5.4	12.2+5.2
Prop-In	13.8+5.6	12.2+5.3	11.1+6.6	10.5+6.8	10.1+6.7	10.6+6.3

\*S.D.





# APPENDIX 13

TABLE 20 - Temperature Differentials ( $T_R - T_S$ ) of Sheep at Summit Metabolism

	Time (hours)					
	0.5	1	1.5	2	2.5	3
Con-Con	25.8+3.1*	25.8+1.5	26.0+2.7	26.0+0.8	25.5+1.0	26.2+0.5
Con-Out	25.5+2.1	25.8+1.3	26.5+1.7	26.5+1.7	27.0+2.7	27.0+2.2
Con-In	27.5+1.7	28.3+2.5	29.0+1.6	29.0+1.6	29.3+1.5	29.3+1.5
Prop-Con	25.0+2.9	26.0+3.4	26.5+2.5	27.0+2.2	28.0+2.0	27.5+2.1
Prop-Out	24.5+4.5	25.3+4.3	25.8+4.8	25.5+4.5	26.3+4.3	26.3+4.3
Prop-In	26.3+4.9	26.3+5.6	27.0+6.8	27.5+7.1	28.0+6.9	28.0+6.9

\*S. D.

Mean temperature differentials for sheep subjected to extreme cold stress (-30°C).  
Values are average for all sheep during each 30 minute period of exposure.



TABLE 21. Room Temperatures °C (-30°C)

	Time (hours)						
	0	0.5	1	1.5	2	2.5	3
Con-Con	-24.4+2.3*	-26.7+1.6	-27.3+1.4	-27.4+1.2	-27.7+1.1	-28.2+1.1	-28.3+1.2
Con-Out	-27.6+2.4	-28.9+1.9	-29.3+1.5	-28.5+2.1	-28.9+2.3	-28.6+2.9	-28.8+2.7
Con-In	-25.7+4.5	-27.8+2.5	-28.9+2.5	-28.9+2.5	-29.0+2.5	-29.5+2.1	-29.6+2.2
Prop-Con	-23.8+2.4	-26.5+1.7	-27.1+1.2	-27.3+1.1	-27.7+0.7	-27.8+0.5	-27.9+0.5
Prop-Out	-25.9+1.3	-28.3+1.4	-28.8+1.2	-29.1+1.2	-29.9+0.8	-30.0+1.2	-30.3+1.1
Prop-In	-24.9+3.8	-25.3+2.8	-26.0+2.3	-26.7+2.3	-27.3+2.1	-27.6+1.8	-27.5+1.9

\*S.D.

Mean room temperatures (-30°C). These temperatures are 30 minute averages for respective sheep.



APPENDIX 15

Mean Squares - Experiment 3

Summit Metabolism

Source	d.f.	Heart Rate M.S.	Heat Production M.S.
1	1	51,538****	57695.0****
2	2	4,992****	7758.8****
3	5	1,427***	5065.0****
Error	27	299	256.8
Total	35		

1. With and Without propranolol

2. Environmental groups

3. Time periods

Probability of significant difference:

\*\*\*\* 0.1%

\*\*\* 0.5%

\*\* 1.0%

\* 5.0%











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